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MODIFICATIONS OF THE SLIDE-GERMINATION METHOD OF EVALUATING FUNGICIDES INCLUDING THE USE OF VENTURIA INAEQUALIS AND PHYTOPHTHORA INFESTANS[1]

HAROLD J. MILLER

(Accepted for publication October 1, 1948)

Reddick and Wallace (34) first pointed out that the method of using a spore suspension on dried spray deposit "more nearly simulates natural conditions than that of using a drop of the spray substance direct". Attempts have been made to use a dried deposit on slides but usually such an elaborate apparatus is required that it has been more practical to do the preliminary testing directly in the drop of spray suspension without any drying. A simplified method was developed in this study for using a dried spray film. Procedures were developed for growing and using the spores of the apple scab organism (*Venturia inaequalis* (Cke.) Wint.) and potato late blight organism (*Phytophthora infestans* Mont. DeBary); in addition, the techniques for using the brown rot organism (*Sclerotinia fructicola* (Wint.) Rehm.) were modified. Attention was also given to a study of the role of certain materials in spore germination and dosage-response with these organisms as well as with *Alternaria oleracea* Milbraith (18).

GENERAL TECHNIQUE

Surface. Glass slides have been used extensively and are recommended (1) for this type of test. However, as pointed out by Horsfall et al. (12), drops of water do not spread uniformly on glass surfaces. They described a method of coating the slides with cellulose nitrate which gave drops of uniform diameter (7.5 mm.). Cellulose nitrate sheets sold under the name of Pyralin (9) give drops of the same size as the coated glass slides. The sheets can be easily cut to the standard size (3 × 1 in.).

A comparison of check slides using *Sclerotinia fructicola, Venturia inaequalis,* and *Phytophthora infestans* showed no significant difference in spore germination between glass, glass coated with cellulose nitrate, and Pyralin surfaces (Table 1). Tenacity coefficients as determined by spore germination on spray deposits made according to the method of Frear (9) before and after weathering were not significantly different on these three surfaces (Table 1).

Application of standard deposit. As pointed out by McCallan and Wilcoxon (22) the difficulty involved in obtaining a known uniform deposit

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is one of the principal sources of error in the laboratory assay of fungicides. After critically testing several methods for uniformity of deposit, McCallan and Wilcoxon (22) recommended a settling tower. Horsfall et al. (12) described a horizontal sprayer for this purpose. Montgomery and Moore (29, 30) and Young (40) avoided the use of a spray apparatus by pipetting measured amounts of suspension into a standard circle scratched on a glass slide. Peterson (33) modified this method by using a round cover slip pressed into petrolatum coating on a glass slide. The fungicide was applied to this standard area. The amount applied was measured by the number of drops.

The method employed in these studies utilized a circle one square centimeter in area drawn on the slide with a wax pencil which was found to adhere very readily to the Pyralin surface. Ten circles can easily be drawn on a 1×3 inch slide. The area is covered with 0.1 ml. of fungicide suspension from a graduated pipette. Slight agitation of the pipette tip is necessary to spread the suspension over the entire area. The ordinary procedure is to make up the highest concentration to be used and to make a series of dilutions according to recommended ratios (1, 2). A 0.1-ml. sample of each dilution is pipetted into duplicate circles as soon as prepared and a sufficient quantity is retained for the next dilution, the remainder being discarded.

By dividing the percentage concentration in the fungicide suspension by 1000 a figure is obtained which expresses the amount of fungicide in micrograms per square centimeter. Multiplying this value by 10 will give a reading in parts per million assuming that all of the deposit exerts fungicidal action when re-wetted with the spore suspension.

Application of spore suspension. Correlated with the necessity for a uniform deposit is the problem of applying the spore suspension on a known area. The amount of deposit covered by the spore drop has been found to be an important variable by Horsfall et al. (12) and methods involving the spraying of the whole slide do not make provision for standardizing this area. Where the area is limited by a circle the amount of spore suspension has been standardized by applying a given number of drops (33) or a measured quantity (29, 30, 40).

In these studies the spore suspension was applied with a graduated pipette also. Exactly 0.1 ml. of suspension was placed on the dried fungicide so that the deposit was diluted to its original concentration. Thus only 2 ml. of spore suspension were required for each material being evaluated.

Incubation of spores. The recommended method (1) suggests the use of moist chambers inverted and sealed with water. Young (40) stacked Petri plate lids separated with glass plates and covered them with a bell jar. Culture dishes (150 or 200 mm. $\times$ 20 mm.) gave sufficiently high humidity for germination and were comparable to larger moist chambers. No loss in water could be detected even though they were not inverted and sealed with

TABLE 1.—*The effect of certain variable factors on percentage germination, LD50 values on a Bordeaux deposit, and coefficients of variation expressed in terms of statistical significance. Six replications on different days.*

Factor	Substances added	*Sclerotinia fructicola*	*Venturia inaequalis*	*Phytophthora infestans*	*Alternaria oleracea*
Juice concentration	Orange juice	**[a]	**	.....	.....
Replicate experiments	Do	**	**	**	.....
24 vs. 48 hours	Do	**	**	**	.....
Replicate drops	Do	none	*[b]	**	.....
Juice lot	Do	**	**	.....	.....
Germination surfaces[c]	Do	none	none	none	.....
Tenacity coefficient of surfaces[c]	Do	none	none	.....	.....
Extract concentration	Lima bean	.....	.....	**	.....
Citrate concentration	K & Na citrate	**	**	**	**
K vs. Na citrate	Do	none	**	*	**
Replicate experiments	Do	*	**	**	**
Sucrose[d] vs. no sucrose	Do	none	.....	*	*
Sucrose concentration	Do	none	.....	**	.....
Age of culture	Do	.....	.....	**	.....
Coefficient of variation	Do	20.7	14.1	13.4	16.7
Do	Bean, juice[e]	16.7	12.6	13.2	.....
Do	Dextrose	113.8	.....	.....	.....

[a] ** indicates significance at odds 99: 1.
[b] * indicates significance at odds 19: 1.
[c] Glass vs. coated glass vs. Pyralin.
[d] Actually 0.5 per cent sucrose.
[e] Difco lima bean extract—*P. infestans;* Orange juice—*S. fructicola; A. oleracea.*

water. Filter paper had no inhibiting effect on germination as compared to water only in the bottom of the dish.

The recommended method (1) specifies temperatures of 20° to 25° C. for spore germination. All spores were held at 18° C. for germination tests reported here unless otherwise indicated. As noted in table 1, 48 hours at this temperature gave a significantly different LD50 value from those held only for 24 hours. This effect of time has also been studied by Wellman and McCallan (38). In these studies the recommended (1) period of 20 to 24 hours was used at all times.

SPORE PRODUCTION

Sclerotinia fructicola and Alternaria oleracea.[2] The method of culturing and producing spores of these two fungi is fairly well standardized (1). Considerable variation was experienced in the yield of *S. fructicola*[3] on the recommended potato-dextrose agar and yields were not so high as those obtained by McCallan et al. (20). Sporulation was much higher on malt agar (Table 2) and more uniform (28). The 10 per cent malt gave the best yield.

Venturia inaequalis.[4] Reddick and Wallace (34) who first used *V. inaequalis* in laboratory slide tests, and several others (6, 24, 25, 37), obtained spores from leaves. Montgomery and Moore (30) produced the conidia on sterilized apple twigs. The same authors (29) also used a thin layer of malt agar to coat the inside of a test tube. Palmiter (31) and Keitt and Palmiter (15) described a method of producing conidia on a cheesecloth wick dipped in malt extract solution.

Since *Venturia inaequalis* does not grow extensively when transferred with a needle, it was necessary to use a method which would produce a uniform growth over the whole surface of the media in order to obtain maximum spore production per tube. This was accomplished by adding sterile water to a tube of the culture and scraping the surface with a transfer needle to make a spore suspension. This suspension was poured from one tube of media to the other. Sufficient numbers of spores lodge on the agar surface to give a good growth over the whole surface.

Relatively little attention has been given to factors influencing sporulation of *Venturia inaequalis* in culture. Montgomery and Moore (30) found 14 days to be optimum for production of conidia on twigs in culture tubes. Keitt and Palmiter (15) obtained good conidial yield in 10 days at 16° C. The number of conidia obtainable in water by rubbing the agar surface was highest at 21° and 9° C. (Fig. 1) indicating a double maximum curve. Only seven days were required at 21° C., while 25 were necessary at 9° C. Maximum yield was not reached at 6° C. within 35 days.

[2] Cultures of *Sclerotinia fructicola* and *Alternaria oleracea* were obtained from Dr. McCallan, Boyce Thompson Institute, Yonkers, New York.

[3] All spores in this study were removed by rubbing in water with a rubber policeman as recommended (1).

[4] *Venturia inaequalis* was isolated from ascospores discharged from apple leaf onto malt agar.

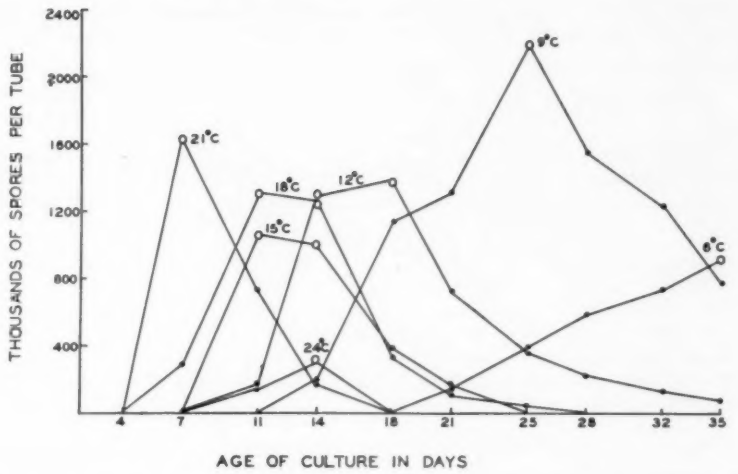

FIG. 1. *Venturia inaequalis.* Relation of age of culture and temperature to yield of conidia on 5 per cent malt agar. Three replicates of two tubes.

Conidial production was also influenced by malt concentration (Fig. 2). Ten per cent malt was best at 15° and 18° C.

Using three different lots of spores consecutively in transferring (three sowings on one tube) gave a significantly higher (approximately one-third) yield of spores than using one lot. Three tubes were grown at three different temperatures (12°, 15°, and 18° C.). Autoclaving the malt agar media longer than 20 minutes decreased the yield of conidia (Fig. 3). Steaming the malt showed no effect as compared with suspending it in hot water before tubing. Concentration of spores at time of transfer had little effect on conidial yield provided there were 200,000 or more per ml. Riboflavin (0.0125 to 0.1 per cent) had no effect on number of conidia per tube.

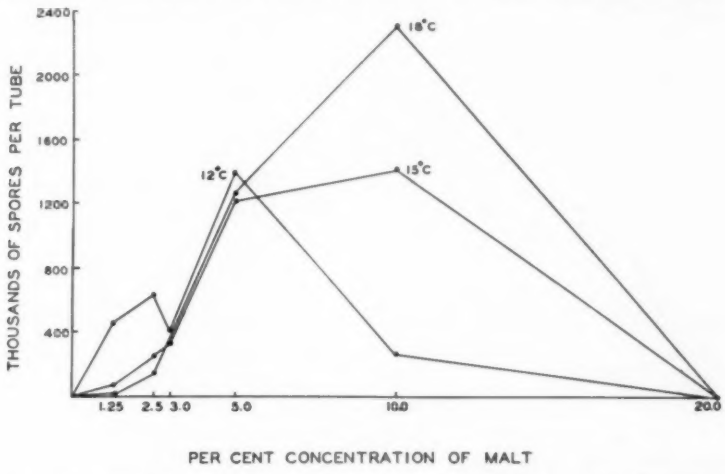

FIG. 2. *V. inaequalis.* Malt concentration and conidial production at different temperatures. Six replicates of two tubes at 14 days.

TABLE 2.—*Yield of conidia of Sclerotinia fructicola on different concentrations of malt, Difco malt, and potato-dextrose agar. Six replicates of two tubes at seven days*

Agar media	Percentage Malt	Thousands of spores per tube
Potato dextrose[a]	.....	1431.3
Potato dextrose[b]	.....	4516.5
Malt	1.25	2439.5
Do	2.50	3814.6
Do	5.00	4891.0
Do	10.00	6408.4
Do	20.00	3222.9
Malt (Difco)	1.00	1560.4

[a] 200 gm. potatoes and 20 gm. dextrose.
[b] 100 gm. potato ground in blender and all added to media without heating previous to autoclaving.

Phytophthora infestans.[5] The fact that pure cultures are required for the slide germination technique precluded the use of the raw potato disc method developed by Crosier (4). Miller (28) found that addition of various materials, including vitamin C (32), to potato-dextrose agar did not give a sporangial yield equal to that of Lima bean agar. The latter was first used by Clinton (3) and was made by steaming 100 gm. of ground Lima beans in 1000 ml. of water and filtering through cheesecloth or a coarse sieve. This was added to 400 cc. of water in which 17 gm. of agar had been dissolved. The final volume was adjusted to 1000 cc., tubed, and autoclaved. Cultures have been carried for several years on this medium.

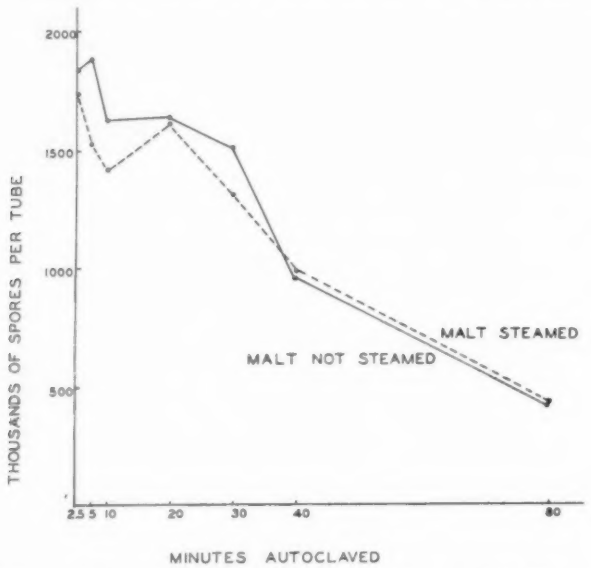

FIG. 3. *V. inaequalis.* Time of autoclaving 5 per cent malt agar and yield. Six replicates of two tubes in 10 days.

[5] *Phytophthora infestans* culture was obtained from a potato tuber from W. R. Mills of this station and is designated as the AAB_2 race.

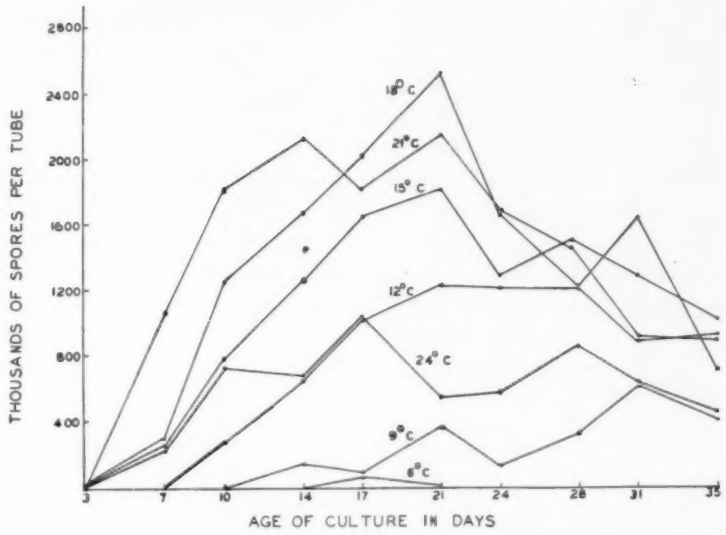

FIG. 4. *Phytophthora infestans.* Yield of sporangia and age of culture at different temperatures. Three replicates of two tubes.

Seventeen to twenty-one days were required for cultures to give highest yield at the optimum temperature of 18° C. (Fig. 4 and 5). Clinton (3) also found growth best around 19° C. Sniezko et al. (35) reported yields of sporangia on peanut hull grain media up to 2,000,000 per gm. dry weight

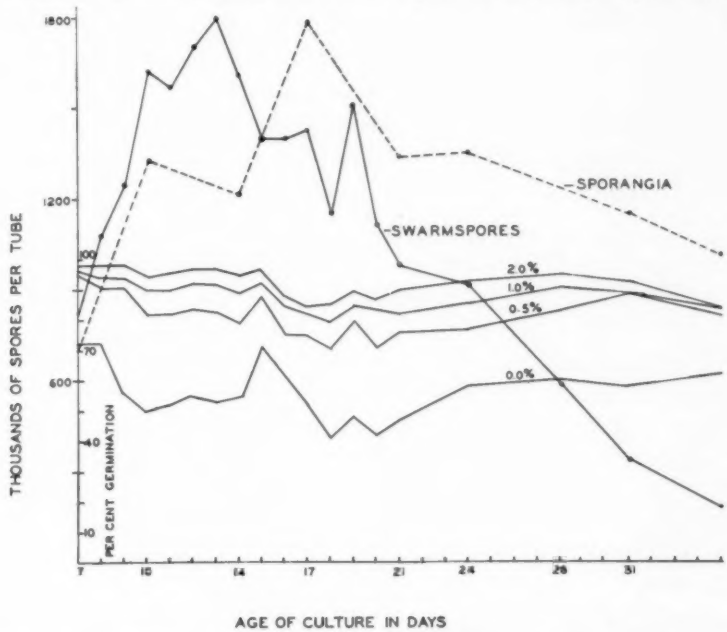

FIG. 5. *P. infestans.* Age of culture grown at 18° C. and yield of sporangia and swarmspores. Also relation of culture age to germination of swarmspores at different sucrose concentrations and 0.005 per cent K citrate. Six replicates of two tubes.

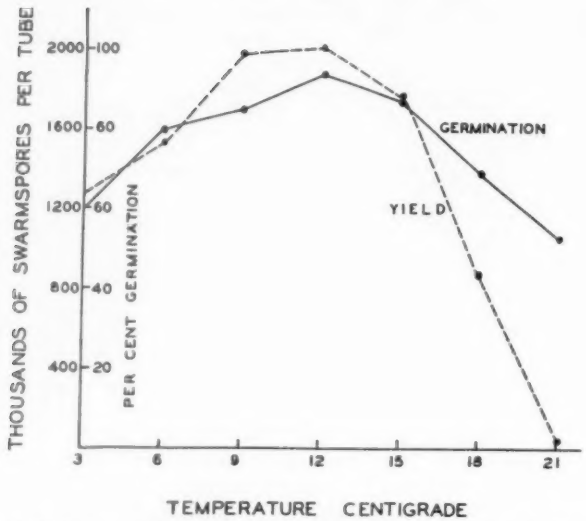

FIG. 6. *P. infestans.* Temperature at which swarmspores are formed, yield and germination. Six replicates of two tubes.

which is comparable to the 2,500,000 at 18° C. on the Lima bean agar (each tube contained approximately one gram dry weight).

Swarmspores form readily if the sporangial suspension is merely placed at a favorable temperature, as found by Melhus (26) and Crosier (5). The optimum temperature for germination of centrifuged sporangia was 12° C. (Fig. 6) which corresponds very closely to that found by these workers.

Crosier (5) obtained data which seemed to indicate that bentonite increased the germination of sporangia obtained from potato tubers. A statistically significant reduction in swarmspore yield was caused by the addition of bentonite to centrifuged suspensions of sporangia in glass-distilled, ordinary distilled,[6] and tap water (Table 3). No increase in sporangial

TABLE 3.—*Yield and germination of Phytophthora infestans swarmspores in different kinds of water with and without bentonite. Sporangia at 12° for three hours. Average of six replications*

Kind of water	Swarmspores per tube	Germination	
		No nutrients	K citrate + Sucrose[a]
	Thousands	*Per cent*	*Per cent*
Distilled	2,847	3.5	95.2
Do + Bentonite	1,845	.....	.....
Tap	2,800	39.9	95.2
Do + Bentonite	2,448	.....	.....
Glass distilled	3,418	64.9	93.3
Do + Bentonite	2,391	.....	.....

[a] 0.005 per cent K citrate and 0.1 per cent sucrose.

[6] Ordinary distilled water was obtained from a metal still with a storage tank lined with tin.

germination resulted with vaseline (4, 36). Yield was highest in glass distilled water.

The time required for maximum swarmspore yield was found by Crosier (5) to be three hours at 12° C. McCallan and Wellman (19) found six hours were required at 10° C. In these studies maximum yield of swarmspores was secured in three hours in a 5-ml. suspension at 12° C. (Fig. 7). After this time the number that could be recovered decreased because of loss of motility and consequent settling to the bottom of the dish. These could not be dislodged by moderate agitation of the suspension. Amount and temperature of water used to make the sporangial suspension will no

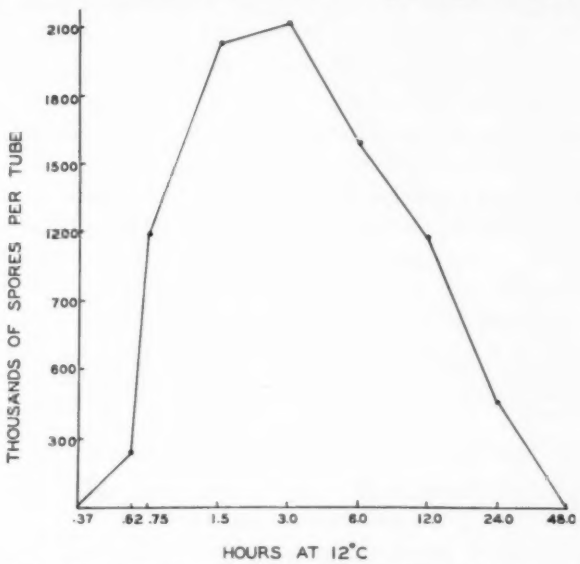

FIG. 7. *P. infestans.* Time at 12° C. and swarmspore yield of a 5-cc. suspension. Seven replicates of one tube.

doubt influence the time required. Three hours at 12° C. was used as a standard unless otherwise indicated.

Age of the original culture also influenced the formation of swarmspores (Fig. 5); cultures 10 to 14 days old gave the highest yield at 18° C. Ten days was used as a standard age. Agitation of the original suspension when removed from the culture tube increased the yield of swarmspores, a factor which must be due to the rapid settling rate of the large sporangia.

Depth of water in the germinating dish influenced swarmspore yield. Approximately one-third more swarmspores were obtained in 0.75 inch of water than in 2.5 inches. Five to ten ml. of water in a small (30 × 50 mm.) preparation dish has been satisfactory for each tube of *Phytophthora infestans.*

Factors that were varied but did not influence swarmspore formation were as follows: length of time water was in original culture tube; concentration of sporangia; temperature (6°–20° C.) of water used to remove

sporangia; length of time of centrifuging; number (0–3) of washings of sporangia by centrifuging; addition of sucrose.

SPORE GERMINATION AND DOSAGE RESPONSE

The conditions necessary for germination of fungus spores in water has received considerable attention (5, 6, 7, 8, 14, 26, 39). Goldsworthy and Green (10) found that coenzyme R would give good germination of *Sclerotinia fructicola*. McCallan and Wilcoxon (21) and Peterson (33) pointed out that varying amounts of media on which this organism was grown were removed in the process of making the spore suspension and were related to the varying percentage of germination. Peterson found an extract of Difco potato-dextrose agar would insure satisfactory germination. McCallan and Wilcoxon (21) eliminated this variable by centrifuging the spore suspension and adding a standard concentration of orange juice. The standardized procedure (1) recommends 0.1 per cent ultra-filtered orange juice for *S. fructicola* and *Alternaria oleracea*.

The importance of glass-distilled water was studied by Lin (16) and Marsh (23). With *Sclerotinia fructicola*[7] (Table 4), glass-distilled water gave significantly (99:1) better germination on check slides than ordinary distilled water with only Bacto-dextrose added. With *Phytophthora infestans* (Table 3) ordinary distilled water alone allowed almost no germination of swarmspores while with glass-distilled water over 50 per cent germinated, and with tap water germination was intermediate between these two.

Sclerotinia fructicola requires some additional substances after washing[8] the spores to give a range of 90 per cent or better, germination (Table 4) as recommended in the standardized method (1). Lin (16) found that 0.5 per cent purified dextrose permitted satisfactory germination. This was substantiated (Table 4), although no difference was found between purified (Difco-Bacto) and the technical grade of dextrose. $MgSO_4$, $CaSO_4$, and KH_2PO_4 were tried, but only $MgSO_4$ (0.1–20 millimols per liter) with 0.5 per cent sucrose gave the required increase in germination. Finally, potassium or sodium citrate and sucrose were tried and gave satisfactory germination (Table 4). Malic and citric acids also gave the same response with sucrose (Table 4). Washed spores of *Venturia inaequalis* and *Alternaria oleracea* also require added substances to give the required percentage germination and responded to the K and Na citrates and sucrose (Table 4). *A. oleraceae* was inhibited by malic and by citric acids with 0.5 per cent sucrose.

Phytophthora infestans swarmspores also failed to germinate satisfactorily when washed by centrifuging (Table 4). No response was obtained with orange juice (0.1 per cent) or with riboflavin (0.001 per cent). Malic acid and citric acid with 1.0 per cent sucrose inhibited germination.

[7] All germinations on slides were made at the recommended (1) spore concentration of 50,000 per ml.

[8] All spores were washed by centrifuging (1).

TABLE 4.—*Percentage germination of washed spores on check slides. Average of six replications except malic and citric acids*

Substances added	Distilled water	*Sclerotinia fructicola*	*Venturia inaequalis*	*Alternaria oleracea*	*Phytophthora infestans*
None	Ordinary	46.2	83.7	64.6	3.5
None	Glass	42.1	.....	.....	64.9
0.1 per cent Tech. Dextrose	Do	89.9	.....	.....	.....
0.1 per cent Bacto-Dextrose	Do	87.7	92.5	.....	.....
Do	Ordinary	54.4	93.3	.....	.....
K citrate[a]	Do[b]	64.2	90.1	77.0	73.8
Sucrose[a]	Do[b]	80.8	94.8	73.6	88.9
K citrate + Sucrose[c]	Do[b]	99.0	95.2	96.8	95.8
Na citrate + Sucrose[c]	Do[b]	95.8	96.3	97.8	42.8
0.1 per cent Orange juice	Do	95.7	95.8	75.3	71.1
0.1 per cent Difco extract	Do	.....	.....	.....	95.7
0.001 per cent Malic acid[c]	Do[b]	91.0	94.8	0.0	9.0
0.001 per cent Citric acid[c]	Do[b]	97.8	92.3	3.1	0.0

[a] *S. fructicola, V. inaequalis, A. oleracea*—0.001 per cent K and Na citrate and 0.1 per cent sucrose; *P. infestans*—0.005 per cent K citrate and 2.0 per cent sucrose.

[b] Glass distilled for *P. infestans.*

[c] *S. fructicola, V. inaequalis, A. oleracea*—0.5 per cent sucrose; *P. infestans*—1.0 per cent sucrose.

With Difco Lima bean agar extract (0.1 per cent) there was 95 per cent germination; and with 0.005 per cent potassium citrate and 2.0 per cent sucrose germination also was satisfactory (Table 4). Germination was related to concentration of sucrose (Fig. 5) with 2.0 per cent of the sugar bringing about the best response.

Age and temperature at which spores are grown influence the percentage germination (17, 20). McCallan (17) suggested that the optimum temperature for spore production of *Venturia inaequalis* was different from that for best spore germination. At 18° C., 90 per cent or more of the spores of *Venturia inaequalis* from cultures 7 to 14 days old germinated; at 15° C., as high a percentage germination occurred with spores from cultures 11 to 14 days old; and at 12° C., with spores from cultures 11 to 18 days old (Fig. 8). *Phytophthora infestans* (Fig. 5) swarmspores germinated best at 18° C. from cultures 7 to 15 days old.

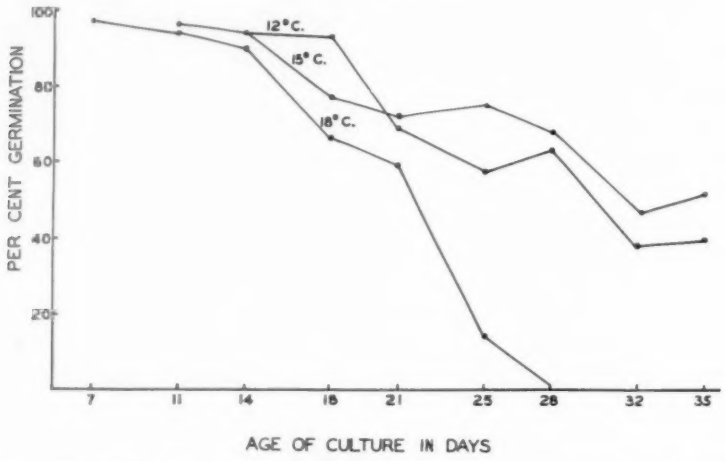

FIG. 8. *V. inaequalis.* Age of culture at different temperatures and conidial germination. Six replicates of one tube.

Temperatures at which swarmspores of *Phytophthora infestans* are formed have an effect on germination at 18° C. (Fig. 6). Those formed at 12° C. gave the highest percentage germination.

Henry and Smith (11) found that sufficient chromate remained on glassware cleaned with sulfuric acid-dichromate mixture to be toxic to microorganisms even after many rinsings with water. No evidence could be obtained for quantities remaining on glassware sufficient to inhibit germination of *Phytophthora infestans* swarmspores.

Other factors that did not influence germination of this organism were: centrifuging sporangia one to three times; holding sporangial suspension at 12° C. for 45 min. to six hours; concentration of swarmspores; diluting one to ten minutes after removal from 12° C.; temperature of water to dilute swarmspores, and type of water in which glassware was rinsed.

Miller (27) pointed out the linear relationship between increasing concentration of orange juice or citrate and higher LD50 values on a Bordeaux

mixture deposit. This emphasizes the importance of standardizing the concentration of these added substances. Also reported by the same author and noted also in table 1 was the variation in LD50 resulting from juice from different lots of oranges which led to the use of the pure chemicals, sodium and potassium citrate. Dextrose alone will insure good germination of *Sclerotinia fructicola*, but the LD50 values on Bordeaux mixture deposits not only failed to come within the range obtained with the standardized procedure using orange juice but also gave a much higher coefficient of variation (Table 1) as a result of a more gradual slope (13).

The role of nutrients or so-called "stimulants" has been the subject of considerable discussion. Lin (16) and Marsh (23) suggested that an increased germination with plant extracts was due to the neutralization of toxic materials in ordinary distilled water by salts that inactivate heavy metals and by proteins that would be contained in these decoctions. The effectiveness of pure citric acid with *Sclerotinia fructicola* would seem to exclude this theory. McCallan (correspondence, 1947) did use glass-distilled and not the ordinary distilled water, as suggested by Marsh, and thus the effect with orange juice could not be explained as a neutralization of toxic ions in ordinary distilled water.

Lin (16) reported that the LD90 of copper sulphate for spores of *Sclerotinia fructicola* was of the order of 0.3 p.p.m. using dextrose only. This agrees with a value of 0.42 p.p.m. on dried Bordeaux deposits found in the experiments using only dextrose with this organism. Where the citrates or orange juice was used, values, of course, were much higher.

STANDARDIZED PROCEDURES DEVELOPED IN THIS STUDY

The fungicide suspension (0.1 ml.) is placed in circles one square centimeter in area drawn with a wax pencil on Pyralin slides. In this area 0.1 ml. spore suspension is placed on the dried deposit and the spores are incubated in a large culture dish.

Sclerotinia fructicola is grown on 10 per cent malt agar at 18°–20° C. *Venturia inaequalis* is grown on 10 per cent malt for 10–12 days at the same temperature and is transferred by a mass sowing of a sterile water suspension of conidia. *Phytophthora infestans* is grown on Lima bean agar for 10 to 14 days. *P. infestans* swarmspores used for germination are secured by placing centrifuged sporangia at 12° C. for three hours in glass-distilled water less than one-half inch deep.

Potassium citrate (0.001 per cent) and sucrose (0.1 per cent) are used in place of orange juice for germination of centrifuged spore suspensions of all organisms except *Phytophthora infestans* which requires these substances at 0.005 per cent and 2 per cent respectively.

SUMMARY

Standardized procedures were developed for the use of *Venturia inaequalis* and *Phytophthora infestans* in slide germination evaluations of

fungicides. Variations in the methods for *Sclerotinia fructicola* and *Alternaria oleraceae* were devised.

Venturia inaequalis produced highest conidial yields at 9° C. in 25 days and at 21° C. in 7 days. The number of conidia that could be removed with water decreased markedly with age in *V. inaequalis*. Swarmspore yield in *Phytophthora infestans* also decreased with age of culture.

When used as a nutrient, orange juice from different lots of oranges gave variable LD50 values. Dextrose and glass-distilled water with *Sclerotinia fructicola* gave very flat dosage response curves with consequent lower and more variable LD50 values.

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OCCURRENCE OF THE BOTRYTIS DISEASE OF GLADIOLUS IN THE UNITED STATES IN RELATION TO TEMPERATURE AND HUMIDITY

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The Botrytis disease of gladiolus as described by Hawker (7) and Moore (14) in England, Timmermans (15) in Holland, Drayton (6) in Canada, and Wade (18) in New South Wales has two distinct phases: corm rot in storage, and a leaf and flower spot and collar rot in the field. This same disease occurs in the United States where it frequently becomes a serious problem in the commercial culture of gladiolus in Florida, California, Oregon, and Washington. Until recently, little attention has been given this disease in the United States although Drayton reported its interception in Canada as early as 1927 (6) on some corms grown in Oregon. According to McWhorter (12), a strain of *Botrytis cinerea* Pers. attacked maturing gladiolus foliage near Portland, Oregon, in 1939. Recently he has written us that "soft rot, due to *Botrytis cinerea* and possibly other species, has been the most destructive gladiolus disease in Oregon. Total loss from this disease has exceeded in one year the losses from all other diseases in five years." Dimock (4) reported an epiphytotic of *Botrytis* on gladiolus foliage in Florida in 1940, although he doubted that a specific gladiolus strain of *Botrytis* was involved. Tisdale (17) questioned Dimock's claim that *Botrytis* was the causal agent. In the same year, Weiss (19) reported a Botrytis dry rot of gladiolus corms on Long Island, and Dodge and Laskaris (5) reported it from the same area the following year. Magie (13) recently discussed the occurrence of Botrytis, Stemphylium, and Curvularia leaf spots as they occur in Florida, and we have discussed the prevalence of the Botrytis disease in the United States (11). The Botrytis disease is now the limiting factor in the culture of gladiolus in California (2).

The present paper presents data concerning the effects of temperature and humidity on the development of the disease and correlates this information with the known occurrence of the disease in the United States.

THE ORGANISM

Klebahn (8) described *Botrytis gladioli* Kleb. in 1930, and Timmermans (16) described *B. gladiolorum* Timmermans in 1942. *Botrytis gladioli* differs from *B. gladiolorum* mainly in the shape of the conidia

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which are long and narrow, averaging 10.4 $\mu \times 4.7$ μ, whereas conidia of the latter species are ovoid to subglobose and average 15 $\mu \times 10$ μ. Conidia of the *Botrytis* isolated by Moore (14), by Hawker (7), by Dodge and Laskaris (5), and by us correspond with those of *B. gladiolorum* (conidia from our isolates average 14.7 $\mu \times 9.8$ μ). Wade (17) called the *Botrytis* he studied *B. gladioli,* but presumably it was *B. gladiolorum* since his spore measurements and other morphological evidence agree with Timmermans' organism rather than with Klebahn's. Miss Hawker (7) considered her isolates to be strains of *B. cinerea* but the spore measurements she gives agree with Timmermans' *B. gladiolorum.* Dennis and Wakefield (3) described the perfect stage of a *Botrytis* isolated from a gladiolus corm by W. Buddin as *Sclerotinia Draytoni* Dennis and Wakefield. They described the conidia as narrowly elliptical and 8–16 $\mu \times 5$–7.5 μ. These spore measurements agree with those of *B. gladioli.* Drayton obtained single ascospore cultures from Buddin and in a personal communication to us states, "After making a number of measurements, Dr. Groves and I are of the opinion that *Botrytis gladiolorum* is the conidial stage of *Sclerotinia* (*Botryotinia*) *Draytoni,* and that this is the fungus responsible for the early-storage decay." Parallel inoculations of Picardy gladiolus corms with one of Buddin's isolates (163-K) obtained by us from Drayton and with one of our own isolates (213-D) have given similar infection (Table 2). *Botrytis gladioli* is poorly described and no mention is made of its effect on the plant. Timmermans points out that Klebahn mounted his spores in glycerine and his measurements might have been greater had the spores been mounted in water. MacLean (10) has reported gladiolus foliage infection with *B. elliptica* (Berk.) Cke. This infection produces foliage symptoms very much like those produced by *B. gladiolorum.* We consider the *Botrytis* used in our tests to be *B. gladiolorum.*

METHODS AND RESULTS

Foliage Infection

At the Plant Industry Station, Beltsville, Maryland, gladiolus plants were inoculated artificially and kept at different temperatures. For the first inoculation test, Picardy corms were planted, one per pot, in soil in the greenhouse October 2, 1946, and 50 plants were selected for uniformity October 31. Forty of these were inoculated by atomizing a spore and mycelial suspension of *Botrytis* on the foliage with a deVilbiss atomizer. This *Botrytis* was a single spore isolate (213-C) made May 1, 1946, from infected gladiolus grown at Redondo Beach, California. Of 32 single spore isolates made at this time, one produced conidia abundantly, four produced both sclerotia and mycelium, and the rest produced neither sclerotia nor conidia. Eight of the inoculated plants and two of the non-inoculated plants were then placed in each of five temperature chambers (35°, 40°, 55°, 65°, and 75° F.) and the humidity was maintained at, or

near, saturation by surrounding the plants with wet cheesecloth. Two inoculated plants were removed from each temperature to a greenhouse maintained at 70° F. daytime and 60° F. night, after each of the following intervals: 24, 72, 120, and 168 hours. The noninoculated plants were removed after 168 hours. In another test, pairs of Picardy, Early Rose, and Pacifica varieties of gladiolus plants were inoculated in a similar manner with isolate 213-D and removed from the five temperatures after 24, 48, 96, 144, and 240 hours. Numbers of Botrytis lesions on Picardy are given in table 1. Similar data were obtained from the Early Rose and Pacifica varieties. These lesions (Fig. 1) were typical of those seen in the field. There was slight infection at 75° F.; infection progressed rapidly at 65° F., less so at 55°, 45°, or 40° F., and there was very little at

TABLE 1.—*Botrytis infection of the foliage of the Picardy variety of gladiolus as affected by time and temperature*

Isolate of Botrytis used	Incubation time[a]	Temperature in degrees F.				
		35	45[b]	55[c]	65[c]	75
	Hours	*Number of lesions on two plants*				
213-C	24	2	2	43	34	15
	72	3	77	131	405	3
	120	43	844	429	....[d]	31
	168	97	685	221	....[d]	79
213-D	24	0[e]	1	5	26	9
	48	9	0[e]	16	176	38
	96	30	50	23	804	25
	144	48	75	399	147[e]	16
	240	63	640	195[e]	280[e]	40

[a] After inoculation the plants were kept in a saturated atmosphere at each of the temperatures for the designated periods of time, then moved to a greenhouse maintained at 65° to 70° F. where the humidity was moderate.

[b] Foliage inoculated with isolate 213-C was incubated at 40° F. instead of 45°.

[c] The lesions on plants incubated at 55° and 65° F. were larger than those on plants incubated at 35° or 45° F., and these tended to coalesce without the typical red necrosis, thus reducing lesion counts.

[d] The foliage was nearly dead after 72 hours and accurate lesion counts could not be made.

[e] Number of lesions on one plant instead of two plants.

35° F. Infection was most rapid at 65° F. and the lesions were largest at this temperature. At 35° F. the lesions were small, pin-point necrotic spots, about 1 mm. in diameter. At 40° F. or 45° F. the pin-point lesions tended to coalesce and become larger (3 mm.) after 6 to 10 days. The lesions on the plants at 55° F. were larger than at lower temperatures and tended to coalesce without the typical red necrosis. The entire leaf tips were killed after 120 to 168 hours, which resulted in fewer lesions being counted. After 6 to 10 days, many pin-point lesions developed and these were thought to be due to secondary infection. Twenty-five lesions developed on the noninoculated Picardy plants held at 65° F. for 168 hours. They were probably due to infection from spores blown from the non-

inoculated plants kept in the same chamber. Thus, the optimum temperature for the leaf-spotting phase of the disease is about 55° to 65° F. with slightly less infection at 40° to 45° F. Thirty-five degrees F. is below the point of rapid infection and 75° F. is above it. Consequently, the leaf spot and the collar rot phases of this disease would be expected to be most severe in areas where temperatures are low and humidity is high during the growing season. The disease could also be severe late in the season or during short periods of cool, wet weather. Both Wade (18) and Timmermans (15, 16) obtained optimum growth of the fungus in culture between 68° and 71.6° F., and Wade points out that this is in agreement with

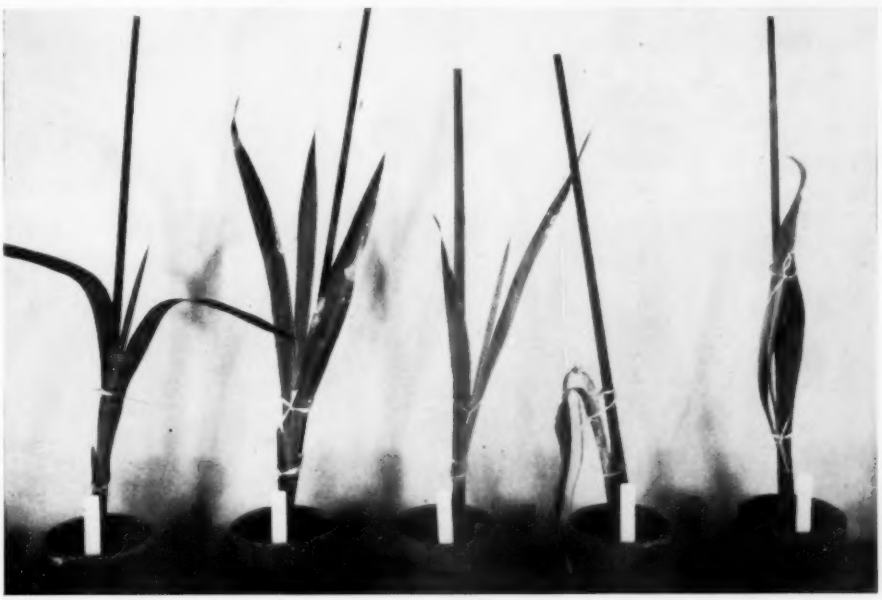

FIG. 1. Picardy gladiolus inoculated with *Botrytis gladiolorum* and incubated in a saturated atmosphere at the following temperatures, left to right, 35°, 40°, 55°, 65°, and 75° F. for 168 hours before removal to a greenhouse maintained at 65° to 70° F. Photographed by Pryor 15 days after inoculation.

field observations that the disease becomes most severe in Australia late in the season when temperatures are low and humidities are high.

Corm Infection

Picardy gladiolus corms were inoculated at Beltsville by cutting them in three places with a scalpel and dipping them in a spore and mycelial suspension of *Botrytis*. These corms were stored in moist chambers at 35°, 45°, 55°, 65°, and 75° F. In one test, 100 corms were dug March 13, 1947, and 20 corms were placed at each of the five temperatures. On March 14, ten corms from each temperature chamber were wounded, inoculated with isolate 213-D, and placed in moist chambers in each of their respective temperatures. The remaining ten corms from each temperature

were wounded similarly and placed in another set of moist chambers as controls. These corms were removed May 6 and examined for rot. They were photographed May 14 (Fig. 2, B). There was no infection apparent at 55°, 65°, or 75° F.; slight infection appeared around the cuts at 45° F.;

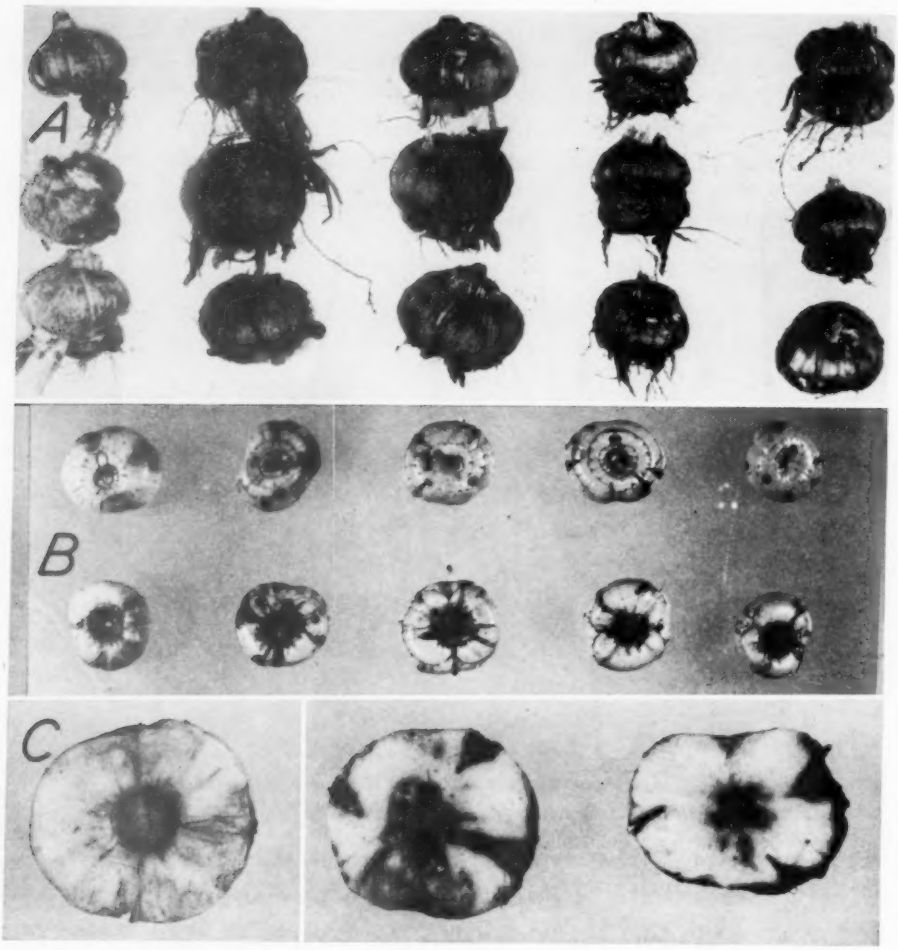

FIG. 2. Picardy corms inoculated with Isolate 213D of *Botrytis gladiolorum* and incubated (A and B), left to right, at 35°, 45°, 55°, 65°, and 75° F., and (C) at 35° left, and 45° F. center and right. Note the lighter color of the rot at 35° and the absence of rot at 65° and 75° F. A, Corms inoculated October 13, 1947, and photographed by Pryor 67 days after inoculation. B, corms inoculated March 13, 1947, and photographed 62 days after inoculation. The husks were removed before photographing. C, cross sections of corms incubated at 35° left, and 45° F., center and right, for 62 days. Photograph by Mead.

and infection was greatest with abundant mycelial growth and sporulation at 35° F. In a second test, corms were dug October 13, 1947, wounded by cutting each corm with a scalpel in three places, and inoculated two hours later by dipping them in a spore and mycelial suspension of *Botrytis*.

Two isolates were used, the 213-D isolate from California and the 163-K isolate from Buddin, 45 corms being inoculated with each isolate. Nine corms inoculated with each isolate were placed in moist chambers and stored at each of the five temperatures. Records were obtained December 23, and these data are given in table 2. Corms were rotted by *Fusarium* at the three highest temperatures and by *Botrytis* at the two lowest temperatures, although there was no rot among corms inoculated with isolate 213-D at 45° F. Mycelium of both isolates grew over the husks of the corms at the three lowest temperatures and sporulated abundantly. Sclerotia formed on corms inoculated with isolate 213-D (Fig. 3) at the three lowest temperatures and on corms inoculated with isolate 163-K stored at

TABLE 2.—*Infection of Picardy gladiolus corms with two isolates of Botrytis gladiolorum. Corms incubated at different temperatures for 67 days after inoculation*

Temperature (° F.)	No. corms with Botrytis fruiting[a]	No. corms with sclerotia	No. corms having rot	Organism isolated
		Isolate 213D		
35	9	7[b]	5	Botrytis
45	9	9	0	do
55	1	1	7	Fusarium
65	0	0	9	do
75	0	0	9	do
		Isolate 163K		
35	9	9[c]	3	Botrytis
45	8	0	1	do and Fusarium
55	3	0	3	Fusarium
65	0	0	8	do
75	0	0	9	do

[a] Nine corms were inoculated with each organism at each temperature.
[b] These sclerotia were immature when examined.
[c] These sclerotia were slightly smaller than those on corms inoculated with Isolate 213D.

35° F. These latter sclerotia were slightly smaller than those from isolate 213-D.

The appearance of those corms inoculated and held at 35° F. was much different from those held at higher temperatures (Fig. 2). Corms held at 45° F. or higher had darkened husks and the rotted areas were very dark, whereas the husks and rotted areas of those held at 35° F. were lighter in color and the rotted areas were not so well defined. This was true of corms inoculated March 14, 1947, and also of those inoculated October 13, 1947. The reason for these differences in color is not known. Hawker (7) points out that, although Moore (14) described three forms of rotting of gladiolus corms associated with species of *Botrytis*, his three forms of rotting may be merely expressions of the same disease as affected by temperature.

In another test in which mature corms were dug from pots in the greenhouse at Beltsville on July 21 and inoculated August 8, no *Botrytis* infection was present after 66 days at any temperature, although some Fusarium rot was present at 55°, 65°, and 75° F. Drayton (6) observed that infection of the corms and spread from corm to corm take place more rapidly at 38° and 45° F. than at higher temperature ranges. Furthermore, in one experiment he was unable to infect cured corms with *Botrytis*, whereas freshly dug corms rotted rapidly following inoculation. Hawker (7) and and Timmermans (15) report that very little corm rot occurs at temperatures above 68° F. even though the optimum temperature for growth of the *Botrytis* is about 70° F. When corm infection is arrested, the lesion

FIG. 3. Mycelium and sclerotia of *Botrytis gladiolorum* on husks of Picardy corm inoculated with Isolate 213D and incubated at 45° F. for 67 days. Photograph by Pryor.

is surrounded by a layer of suberized cells which walls off the disease (6, 9, 18). Lauritzen and Wright (9) compared the results of Artschwager and Starrett's (1) studies of temperature and humidity on suberization and wound periderm formation in gladiolus with their results on the effect of temperature and humidity on corm rot in gladiolus (stated to be due mainly to *Penicillium*). They found that the temperatures or humidities favorable for rapid suberization and periderm formation prevented or inhibited infection. Thus, at temperatures above 71.5° F. suberization took place rapidly and infection was prevented, whereas at low temperatures suberization was much delayed and infection occurred. Corms stored at 40° F. for 79 days had very little wound periderm and at 59.5° F. wound periderm did not form until after 22 days. Suberization also

took place much more slowly at low humidities. The explanation for the low temperature requirements for *Botrytis* infection of gladiolus corms becomes clear in the light of this information. Undoubtedly, if it were not for rapid suberization and wound cork formation at higher temperatures, optimum temperature requirements for corm infection would more nearly parallel those for foliage infection.

Timmermans (15) concluded that rot was much more prevalent on corms dug late in the season than on those dug early. Wade (18) dug corms early, midseason, and late, and the percentage of Botrytis rot was 0.5, 27.1, and 21.7, respectively. Similar results were obtained by Hawker (7). In an experiment in which Picardy corms were dug at two-week intervals beginning September 24, 1946, at Puyallup, Washington, Botrytis

TABLE 3.—*Botrytis rot in Picardy gladiolus corms at Puyallup, Washington, in 1946, as affected by the digging date*

Digging date	Average daily rainfall between digging dates	Mean temperature[a]	Total corms harvested[b]	Corms with Botrytis rot	
	Inches	*° F.*	*Number*	*Number*	*Per cent*
Sept. 24		58.4	1275	31	2.43
	.109				
Oct. 8			1355	39	2.88
	.129	47.5			
Oct. 22			1360	61	4.48
	.053				
Nov. 5			1380	64	4.64
	.222	40.4			
Nov. 26			1352	197	14.57
	.340				
Dec. 16		39.8	1333	368	27.61

[a] These are mean temperatures for the months indicated.
[b] Data are based on the combined totals of two planting dates (April 11 and May 27).

rot was least in the early diggings and greatest in the corms dug last (Table 3). There was a progressive increase in rainfall and decrease in temperature during this time, so that conditions were favorable for development of Botrytis rot and poor for curing corms.

DISCUSSION

If we correlate the local climatic conditions under which gladioli are grown with the conditions that appear to be necessary for *Botrytis* infection, the reason for the prevalence of the disease in certain areas in the United States becomes evident. Corm rot is not a problem in Florida although leaf spotting becomes severe sporadically. In the commercial gladiolus areas of upstate New York and of Illinois, Indiana, and Michigan, *Botrytis* has not been reported on gladiolus. However, it has been reported as causing a corm rot of gladiolus grown on Long Island (2, 10). Dimock reported to us that Botrytis leaf spot occurred late in the fall of

1947 and that Botrytis corm rot was prevalent this past winter (1947–48) in upstate New York. There are no previous reports of the occurrence of this disease in upstate New York. Both the corm rot phase and the leaf spot phase of the disease are severe in Oregon, Washington, and California.

Gladioli are grown in upstate New York, Michigan, Illinois, Indiana, Washington, and Oregon during the summer months only, whereas in the coastal areas of Southern California they are grown commercially as an early spring crop flowering in April and May. In Florida gladioli are grown as a winter crop with the peak of flower production occurring in February and March. Florida gladioli are subject to "northers" during January, February, and March. These usually last for 3 or 4 days and are accompanied by cold, wet weather. Fogs in Florida are confined almost exclusively to the early mornings during the winter. It is during these times that *Botrytis* develops rapidly on the foliage and flowers, resulting in a big decrease in flower quality. Corm rot is not usually a problem in Florida, however, since the average temperature, even in January, in the gladiolus-producing areas is 60° to 65° F., which is sufficiently high to cure the corms rapidly and to wall off infection by suberization before the corms are placed in commercial storage at 40° F. Thus, temperature limits *Botrytis* in Florida to occasional epiphytotics of foliage spotting.

Temperatures during the growing season in the gladiolus areas of upstate New York, Michigan, and Illinois are generally too high to permit epiphytotics of the disease. The average July temperatures in these areas are 70° to 75° F. and these temperatures are accompanied by moderate humidities (average 60 to 80 per cent). Thus, except for wet periods early or late in the growing season, *Botrytis* should not generally be a factor in these areas.

In the coastal areas of California cool weather occurs during early spring when gladioli are grown there with temperatures averaging 54.6° to 62.2° F. from January through May at Los Angeles. This coolness, together with occasional periods of rain or heavy fog plus the general practice of using overhead irrigation, provides ideal weather conditions for the foliage phase of the disease. Gladiolus cannot be grown in this area for winter flowering primarily because of *Botrytis*. Corms are commonly held in cold storage in California. If they are dug when the weather is cool and not cured sufficiently before being placed in storage and if plenty of inoculum is available, the stage is set for an abundance of corm rot. This often occurs.

In western Washington the Botrytis blight of leaves and flowers does not generally become severe until the early fall rains and fogs begin. It is usually negligible during July when temperatures average 64° F. at Seattle, precipitation is only 0.6 of an inch, and the relative humidity is 85 per cent at 8:00 A.M. and 52 per cent at 8:00 P.M. August conditions

are much the same, but in September the average temperature is 59°, precipitation is 1.7, and relative humidity is 89 per cent at 8:00 A.M. and 62 at 8:00 P.M. The average number of hours of sunshine for July and September are 312 and 185, respectively. This average drops to 113 in October and 60 in November with an increase in relative humidity and precipitation, and a decrease in temperature. Total rainfall in June, July, and August totals 2.6 inches as compared to 9.5 inches for September, October, and November. The conditions therefore become progressively more favorable for development of the disease. The progressive increase in foliage and flower infection is also reflected in increased corm infection.

In the past many western Washington growers have carried bulbs in common storage, but as a result of the rapid increase in severity of the *Botrytis* corm rot, most of the commercial growers have obtained equipment and designed storage facilities so that corms can be dried rapidly after digging. This is supplemented by culling, treating before planting, cutting and burning of unsold spikes, and in some cases by spraying plants with Bordeaux.

In contrast to the common occurrence of Botrytis corm rot in western Washington under its cool, moist conditions is the relative absence of this disease in eastern Washington, under drier, warmer summer and fall conditions. Most of the limited commercial acreage in eastern Washington is concentrated around Sunnyside, in the Yakima Valley. July temperatures average 71.7° F., with a precipitation of 0.20 inches. September temperatures average 61.0° F., with 0.54 inches of rainfall. Relative humidity data are not available for Sunnyside but at Yakima they are 52 and 20 per cent (8:00 A.M. and 8:00 P.M., respectively) in July, and 67 and 33 per cent in September. An examination of stocks of three of the largest growers in this area in the winter of 1947 failed to disclose a single case of Botrytis corm rot, even though the corms had been held in barn or cellar storage under moist conditions since digging.

It would appear from the foregoing that the occurrence and prevalence of both phases of the Botrytis disease of gladiolus could be predicted with the aid of weather records. If, for instance, a new area were being considered for gladiolus production and it was found on consulting the weather records that the temperatures in this area averaged between 55° and 65° F. during a four-month growing period, and if these temperatures were accompanied by abundant rainfall or heavy fogs, gladiolus production would most likely be very hazardous because of *Botrytis*. If, on the other hand, these same temperatures prevailed but were not accompanied by frequent rain or foggy weather, *Botrytis* would not be expected to be hazardous to gladiolus production in the area. Similarly, if temperatures averaging 70° F. or more prevailed later in the season, gladiolus could then be grown with safety.

SUMMARY

Infection of the foliage of three varieties of gladiolus with *Botrytis gladiolorum* Timmermans under controlled temperature conditions was most severe at 55° and 65° F. Thirty-five degrees was below the point of rapid infection and 75° F. was above it.

The optimum temperature for infection of freshly harvested corms was 35° F. Some infection also took place at 45° F., but none at 55° F. or higher. Infection did not occur on corms that had been allowed to cure for 18 days. Lack of infection of cured corms and of those inoculated and incubated at high temperatures was assumed to be due to the rapid suberization and wound periderm formation that took place at the higher temperatures.

Corms inoculated and incubated at 45° F. or higher had darkened husks and the rotted areas were very dark, whereas the husks and rotted areas of those held at 35° F. were lighter in color and the rotted areas were not so well defined.

Similar corm infection, sporulation, and sclerotial formation occurred from inoculations made with a single spore isolate of *Botrytis* from gladiolus from California and with a single ascospore isolate of *Sclerotinia Draytoni* Dennis and Wakefield.

The percentage of Botrytis rot in Picardy corms was least in early diggings and greatest in the corms dug last, when corms were dug at two-week intervals at Puyallup, Washington.

The occurrence of the disease in the United States is discussed in the light of the data presented and it is concluded that the regional prevalence of both the foliage phase and the corm rot phase of the disease in a given area can be predicted with the aid of weather records.

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EFFICACY OF SOIL FUMIGANTS AS AFFECTED BY SOIL TEMPERATURE AND MOISTURE

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INTRODUCTION

There have been numerous references cautioning against the use of soil fumigants when temperatures are low. Godfrey and Young (3) mention that soil treatments are most effective at temperatures above 70° F., and they state, "For best results with chloropicrin, the temperature of the soil at a depth of 6 inches should be warmer than 65°. Rapidity of penetration and killing increases with rises in temperature. . . . Most of the fumigants are inefficient below 45° F." Newhall, Chupp, and Guterman (6) state that a temperature above 65° is "ideal for fumigation." There are numerous other references alluding to the fact that low soil temperatures decrease the efficacy of soil fumigants. However, a search of the literature yields very little experimental evidence in support of this contention. In fact, Parris (9) observed that D-D was effective when used in soil having a temperature of 38° to 40°. Stark (11) found a significant reduction in the efficacy of chloropicrin when the soil temperature at the time of treatment was reduced from 85° to 73°. Newhall and Lear (7), on the other hand, reported that methyl bromide treatment of soil in sealed drums was just as effective at 48° as at 60°. They also reported good results when Dowfume G was used to treat soil at 52°.

Hagan (4) and Hannesson (5) have both studied extensively the movement of carbon disulfide in soils. They found that the soil moisture content very markedly affects the permeability of the carbon disulfide. Hagan states that moisture affects permeability more than any other factor studied and Hannesson reports that the movement of carbon disulfide is 6 to 10 times greater through air-dry soil than through wet soil. According to Stark (11), high moisture markedly reduces the effectiveness of chloropicrin, although it is commonly recommended that soils be moderately moist for fumigation (1, 3, 6).

To gain information on the effect of temperature and moisture on the efficacy of soil fumigants, tests were made in the greenhouses at the Plant Industry Station, Beltsville, Maryland, in 1946 and the results of these tests are presented here.

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METHODS

The tests were carried out in soil temperature tanks of the Wisconsin type in a greenhouse maintained at 70° to 75° F., although late in the spring air temperatures frequently rose to 90°. There were 6 tanks and the following soil temperatures were maintained: 45° to 50°, 55°, 65°, 75°, 85°, and 95°. No cooling system was used in the tanks, however, and with warmer weather the 45° to 50° tank usually ran at 54° to 55° or higher. There was some variation in the temperatures in the other tanks also (see tables 1, 3, and 4). In each tank there were 4 rectangular cans, each 10 inches wide by 18 inches long by 9 inches deep. Forty-six and a half pounds of an air-dry soil mixture consisting of 2 parts of a heavy composted clay loam and 3 parts of sand were put in each can. The moisture level of 2 of the cans in each tank was maintained at 7 to 10 per cent, and the moisture level in the other 2 cans was kept at 17 to 20 per cent. The cans were weighed daily and water was added when needed. Thus, half of each test was carried out in dry soil and half in wet soil.

The three organisms used in the tests were: the aster-wilt Fusarium, *Fusarium oxysporum* f. *callistephi* (Beach) Snyder & Hansen; *Sclerotium rolfsii* Sacc.; and the root-knot nematode, *Heterodera marioni* (Cornu) Goodey.

For each test the aster-wilt Fusarium was grown on sterilized oats in quart jars for approximately 30 days and then ground in an Enterprise meat grinder. A large teaspoonful of this mixture was put in each of 120 cheesecloth bags. *Sclerotium rolfsii* was grown on a modified Tochinai agar in Petri dishes until sclerotia were well formed at which time they were counted into 120 cheesecloth bags, 50 sclerotia per bag. Root-knot-nematode galls on tomato roots were ground in a food chopper and mixed with moist sand and about 2 teaspoonfuls of this mixture placed in each of 120 cheesecloth bags. While this inoculum contained undecomposed root material, the galls and roots were cut into small pieces.

Five bags each of *Fusarium oxysporum* f. *callistephi* and of *Sclerotium rolfsii* were buried in one end of each can and 5 bags containing *Heterodera marioni* were buried in a similar position at the opposite end. Two and a half ml. of the fumigant was placed 3 inches deep in the center of each can and covered with soil. This point of injection was about 7½ inches from the buried bags. No water seal or other seal was used. One bag of each of the 3 organisms was removed at the following intervals after the application of the fumigant: 1, 3, 6, 9, and 13 or 14 days. In some of the tests, bags containing the organisms were buried in the centers of the cans 1, 3, and 6 days after the application of the fumigant to give information on the retention of the fumigant in the soil. These bags were all removed 14 days after the application of the fumigant.

After the organisms were removed from the soil they were tested for viability. The oat mixture containing the Fusarium was placed in 250-ml.

beakers, water added, and the mixture stirred. Roots of 2 young aster seedlings (about 1 month old) of the susceptible variety Super Giant El Monte were dipped in the suspension and planted in pots of steamed soil in the greenhouse. Death of the aster plants indicated viability of the organism (Fig. 1). The sclerotia of *Sclerotium rolfsii* were planted in Petri dishes on moist Mosinee paper toweling. Percentage of germination was the criterion of viability (Fig. 2). Bags containing the root-knot galls were mixed with steamed soil in pots in the greenhouse and squash seeds were planted in the soil. After 6 weeks the plants were removed from the soil, the roots ex-

FIG. 1. Aster seedlings one month after inoculation with samples of *Fusarium oxysporum* f. *callistephi* that had been exposed for one day to Larvacide in the soil at the following temperatures, left to right: 98°, 81°, 70–75°, 57–59°, 46–48°, and 45–56° F. The first four plants in each row were inoculated with *F. oxysporum* f. *callistephi* that had been exposed to Larvacide in dry soil and the other four with *F. oxysporum* f. *callistephi* that had been exposed in wet soil.

amined, and given a relative root-knot index (10). This rating served as a criterion of nematode viability.

The soil fumigants used in these tests were: D-D (a mixture of dichloropropene and dichloropropane), Larvacide (chloropicrin), Dowfume G (10 per cent methyl bromide by volume in a mixture of 3 parts ethylene dichloride and 1 part carbon tetrachloride), and Dowfume W-15 (15 per cent ethylene dibromide by volume in a naphtha thinner).

RESULTS

Larvacide. The effect of temperature on the efficacy of Larvacide is given in table 1. In this experiment the soil in but 2 of the 4 cans in each

temperature tank was treated with Larvacide; the soil in the other 2 was not treated. Bags of inoculum of the organisms were buried in the soil in each of the cans. Thus, information was obtained on the effect of time and temperature on the inoculum in the untreated soil as compared with that exposed to Larvacide.

Larvacide was not effective against the nematodes except at 98° F. Apparently the bags of root-knot inoculum were just outside the effective killing range of the dosage of chloropicrin used, although the effective killing

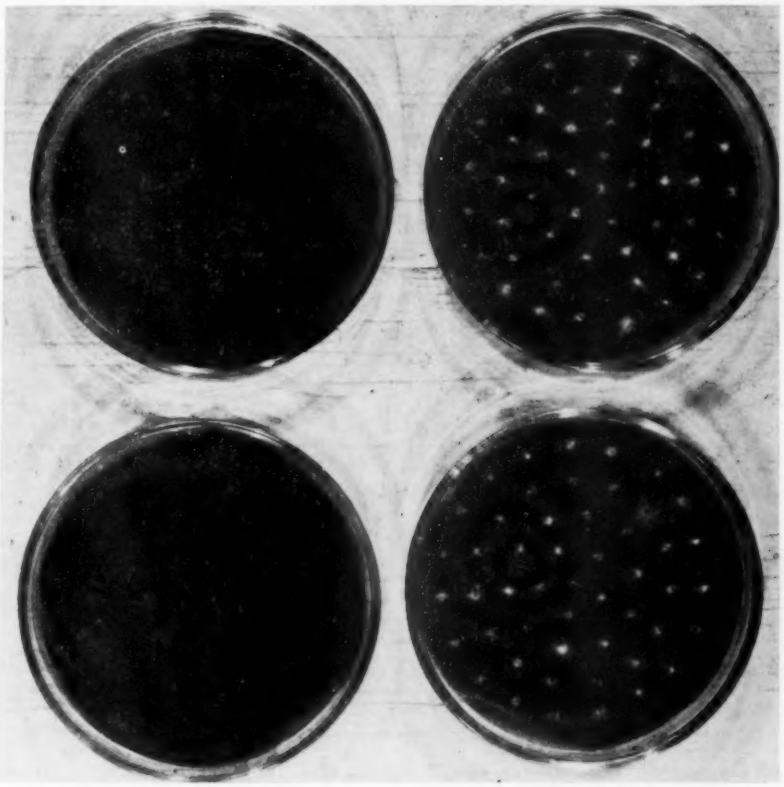

FIG. 2. Germination of sclerotia of *Sclerotium rolfsii*. Left, after 4 days' exposure to Larvacide in soil at 62° F.; right, untreated. Upper plates, wet soil; lower, dry soil.

range of chloropicrin has not been determined for *Heterodera marioni*. Newton, Bosher, and Hastings (8) concluded that 1 ml. of chloropicrin would kill *Ditylenchus dipsaci* at 6 inches while 2, 4, and 6 ml. did not kill all the nematodes at 8 inches. In our tests the bags of inoculum were 7½ inches from the point of injection, a distance that may have been too great for effective kill with chloropicrin.

Another reason for ineffectiveness of chloropicrin may have been the use of unrotted nematode galls. Rotting of galls appears to be necessary for chloropicrin to be effective (12). On the other hand, rotting of galls

TABLE 1.—*Effect of temperature on the efficacy of Larvacide against Heterodera marioni, Fusarium oxysporum f. callistephi, and Sclerotium rolfsii*[a]

Soil treatment	Exposure (in days)	Temperature ° F.						
		52	53	57–59	72	82	98	Totals
		Relative root-knot index[b]						
Larvacide	1	3.0	2.8	2.5	3.0	2.8	0.0	14.1
	3	3.3	3.3	3.0	2.8	2.5	0.5	15.4
	6	3.0	2.8	2.3	1.5	2.0	0.5	12.1
	9	3.5	3.5	3.0	3.0	3.0	0.0	16.0
	14	2.8	3.3	2.3	2.0	3.0	0.0	13.4
	Totals	15.6	15.7	13.1	12.3	13.3	1.0	71.0
None	1	3.7	4.0	3.7	4.0	4.0	4.0	23.4
	3	4.0	4.0	4.0	4.0	3.5	4.0	23.5
	6	4.0	4.0	4.0	4.0	4.0	3.0	23.0
	9	4.0	4.0	4.0	4.0	4.0	4.0	24.0
	14	4.0	4.0	4.0	4.0	4.0	4.0	24.0
	Totals	19.7	20.0	19.7	20.0	19.5	19.0	117.9
		Numbers of dead aster plants[c]						
Larvacide	1	3	4	3	3	4	0	17
	3	4	2	4	3	0	0	13
	6	1	1	2	2	1	0	7
	9	0	1	1	1	0	0	3
	14	1	3	2	2	0	0	8
	Totals	9	11	12	11	5	0	48
None	1	4	4	4	4	4	3	23
	3	4	3	4	4	4	3	22
	6	4	4	4	4	4	0	20
	9	4	3	3	4	4	0	18
	14	3	4	2	4	4	0	17
	Totals	19	18	17	20	20	6	100
		Germination of sclerotia[d]						
Larvacide	1	60	100	86	21	39	20	326
	3	22	14	8	16	15	27	102
	6	15	12	4	2	6	5	44
	9	2	5	6	0	0	54	67
	14	4	0	2	0	2	10	18
	Totals	103	131	106	39	62	116	557
None	1	.....	100	93	97	98	100	488
	3	100	100	100	95	95	99	589
	6	100	97	100	96	99	87	579
	9	97	100	100	100	87	80	564
	14	90	72	64	63	52	52	393
	Totals	387	469	457	451	431	418	2613

[a] Efficacy is based on the viability of these organisms and is tested as follows: relative root-knot index on squash plants, survival of aster plants, and germination of sclerotia, respectively.

[b] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents little or no kill and maximum galling.

[c] 4 dead plants represents no reduction in viability, and 0 dead plants represents complete kill, of the Fusarium.

[d] Each figure represents the number of sclerotia germinating out of 100 tested.

does not appear to be necessary when methyl bromide is used, for Stark and Lear (12) reported that methyl bromide diffused through the soil and killed nematodes in non-rotted galls in 2 hours.

There was a uniform reduction in nematode population at all the lower temperatures in Larvacide-treated soil as compared with the nematode population in the untreated soil at the same temperatures. Apparently the full effect of the Larvacide occurred the first day; no further kill of the nematode was apparent after 3 days or more. Complete kill of the Fusarium occurred at 98° F. after 1 day's exposure and nearly complete kill at 82° after 3 days' exposure. However, a temperature of 98° had a detrimental effect on the Fusarium in the untreated soil, and the combined effect of high temperature and Larvacide may have been responsible for the complete kill at this temperature after 1 day. The reason for the detrimental effect of the high temperature is not clear since this temperature is much below the

TABLE 2.—*Effect of soil moisture on the efficacy of Larvacide against Fusarium oxysporum f. callistephi as indicated by the numbers of dead aster plants*[a]

Exposure (in days)	Soil treated with Larvacide		Soil not treated	
	Wet soil[b]	Dry soil[c]	Wet soil[b]	Dry soil[c]
1	7	10	11	12
3	7	6	11	11
6	3	4	10	10
9	0	3	10	8
14	1	7	8	9
Totals	18	30	50	50

[a] Twelve dead plants represents no reduction in viability, and 0 dead plants represents complete kill, of the Fusarium.
[b] The moisture level of the wet soil was maintained at 17 to 20 per cent.
[c] The moisture level of the dry soil was maintained at 7 to 10 per cent.

thermal death point of most fungi, including Fusarium (6). The effect was less at 72° or lower. At these lower temperatures a longer time was required for effective fungicidal action. Germination of the sclerotia of *Sclerotium rolfsii* was nearly 100 per cent in each of the untreated lots with the exception of those remaining in the soil for 14 days. The majority of these latter sclerotia were in poor condition and had been attacked by other fungi and bacteria which were not identified. Larvacide reduced the viability of these sclerotia at temperatures of 72°, 82°, and 98° after 1 day and at all temperatures after 3 days. Nearly complete kill occurred at all temperatures after 6 days. The discrepancy (Table 1) at 98° after 9 and 14 days could not be accounted for. In another test the number of germinating sclerotia was greater after 14 days' exposure to chloropicrin than similar sclerotia exposed for 6 or 10 days. Godfrey and Young (3) killed sclerotia of *Sclerotium rolfsii* with chloropicrin by using 3.17 ml. per cubic foot of soil in the sealed containers. The temperature was not given. This is approximately the same concentration we used but our treatments were in unsealed containers.

As shown in table 2, Larvacide was more effective against the Fusarium in wet soil than in dry soil, but it was slightly better against *Sclerotium rolfsii* in dry than in wet soil. In two experiments, of 4,500 sclerotia treated in dry soil, 1,138 germinated, and of 4,500 sclerotia treated in wet soil, 1,380 germinated. Davey and Leach (2), however, reported that wet sclerotia of *S. rolfsii* were killed by a 24-hour exposure to chloropicrin, but dry sclerotia were not. Because the root-knot galls apparently were beyond the effective killing range of the Larvacide for this organism in unrotted galls, no information was obtained on the relation between soil moisture and nematode kill.

D–D. Nematode kill was complete after 1 day's exposure to D–D in the soil at 82° to 98° F. and after 3 days' exposure at 62° and 72° (Table 3). D–D was relatively ineffective against *Fusarium oxysporum* f. *callistephi*, although the results were obscured by the fact that the oat medium on which

TABLE 3.—*Relative root-knot index[a] on squash plants following exposure of Heterodera marioni to D-D at different soil temperature and moisture levels*

Exposure (in days)	Soil Temperature (° F.)						Wet soil[b]	Dry soil[c]
	46–54	55	62	72	82	98		
1	3.3	3.5	3.5	3.5	0.0	0.0	2.1	2.5
3	4.0	4.0	0.0	0.0	0.0	0.0	0.8	1.3
6	2.8	2.5	0.0	0.0	0.0	0.0	0.5	1.0
9	2.3	2.1	0.0	0.4	0.0	0.0	0.4	1.2
13	2.9	2.3	0.0	0.8	0.0	0.0	0.4	1.5
Totals	15.3	14.4	3.5	4.7	0.0	0.0	4.2	7.5

[a] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.
[b] The moisture level of the wet soil was maintained at 17 to 20 per cent.
[c] The moisture level of the dry soil was maintained at 7 to 10 per cent.

the fungus was growing fermented badly in the D–D treatments, particularly at the highest temperature (98°). This fermentation did not occur in the other treatments. Because of the fermentation it is questionable to what extent the D–D was responsible for the reduction in viability of the Fusarium; 164 out of a total of 240 aster plants from all D–D treatments were killed, which indicates that the majority of the fungus samples were still viable. In any event, the fungicidal effect was low.

Dowfume G. As in the case of Larvacide, the nematode galls apparently were beyond the effective range of Dowfume G and, although a slight reduction in nematode viability did occur, little effect of temperature or moisture was evident (Table 4). This material was least effective against the nematodes at the highest temperature (90° to 101° F.), contrary to the results with the other fumigants; but there were no differences in effectiveness between the other temperatures. It may be that at temperatures of 90° to 101° the Dowfume G was dissipated from the soil so rapidly that it had no chance to affect the root-knot galls at such a distance.

There was a reduction in germinability of the sclerotia after 6 days' exposure in the Dowfume G-treated soil (Table 4). This reduction was most rapid at the higher temperatures but was not affected by soil moisture. Apparently this reduction was due to the action of antagonistic organisms on the sclerotia, since neither methyl bromide nor ethylene dichloride, constituents of Dowfume G, has been considered to be noticeably fungicidal

TABLE 4.—*Effect of soil temperature and moisture on the efficacy*[a] *of Dowfume G and of Dowfume D-15 against Heterodera marioni and Sclerotium rolfsii*

Soil treatment	Exposure (in days)	Soil temperature °F. 61–70	61–71	64–72	72–80	80–86	90–101	Totals	Wet soil[d]	Dry soil[e]
		Relative root-knot index[b]								
Dowfume G	1	3.0	3.0	3.0	3.5	3.3	4.0	19.8	3.6	3.0
	3	2.5	3.0	2.5	2.8	2.8	4.0	17.6	3.3	2.6
	6	3.5	2.3	2.3	2.8	2.3	4.0	17.2	2.7	3.0
	9	2.5	2.3	2.5	2.5	2.0	3.0	14.8	2.1	2.9
	14	2.5	2.5	2.3	2.0	2.0	3.3	14.6	2.1	2.8
	Totals	14.0	13.1	12.6	13.6	12.4	18.3	84.0	13.8	14.3
Dowfume W-15	1	3.0	3.0	2.3	2.5	2.0	1.8	14.6	2.8	2.1
	3	0.0	0.5	0.5	0.5	0.8	0.0	2.3	0.0	0.8
	6	0.5	0.5	0.5	0.5	1.5	0.8	4.3	0.0	1.4
	9	1.3	1.0	1.0	1.0	1.5	0.5	6.3	0.2	1.9
	14	0.8	1.0	1.3	1.0	1.0	0.0	5.1	0.0	1.7
	Totals	5.6	6.0	5.6	5.5	6.8	3.1	32.6	3.0	7.9
		Germination of sclerotia[c]								
Dowfume G	1	100	100	100	100	100	100	600	300	300
	6	100	95	94	20	35	6	350	151	199
	13	75	67	10	0	21	2	175	95	80
	Totals	275	262	204	120	156	108	1125	546	579
Dowfume W-15	1	100	100	100	89	99	99	587	289	298
	6	94	100	47	59	46	2	348	138	210
	13	51	31	32	0	7	0	121	16	105
	Totals	245	231	179	148	152	101	1056	443	613

[a] Efficacy is based on the viability of these organisms and is tested as follows: relative root-knot index on squash plants and germination of sclerotia, respectively.

[b] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.

[c] Each figure represents the number of sclerotia germinating out of 100 tested.

[d] The moisture level of the wet soil was maintained at 17 to 20 per cent.

[e] The moisture level of the dry soil was maintained at 7 to 10 per cent.

(3), except at high concentrations (7), and most of the sclerotia that failed to germinate were badly disintegrated. Viability of *Fusarium oxysporum* f. *callistephi* was not affected by this material.

Dowfume W–15. This material was effective against nematodes at all temperatures (Table 4). Some reduction in viability was evident after

24 hours' exposure particularly at the highest temperature. No further reduction in viability occurred after 3 days, however. This material was more effective in wet than in dry soil.

Dowfume W–15, like Dowfume G, was not effective against *Fusarium oxysporum* f. *callistephi* but did reduce the viability of sclerotia of *Sclerotium rolfsii*. The greatest reduction occurred at 90° to 101° F., and there was considerable reduction at 64° to 86° after 6 days. Further reduction in viability occurred after 13 days at all temperatures, but how much of this effect was due to the fumigant and how much due to antagonistic organisms is not known. The greatest reduction of viability occurred in moist soil.

Retention of the fumigants in the soil. As mentioned previously, test bags of the organisms were buried in the center of the soil in the cans 1, 3, and 6 days after the introduction of the fumigant, and were removed 14

TABLE 5.—*Effect of moisture on the retention of Larvacide, Dowfume G, and Dowfume W-15 in the soil as indicated by the survival of Fusarium oxysporum f. callistephi and Heterodera marioni introduced into the soil 1, 3, and 6 days after the introduction of the fumigant*[a]

Test organism added after:	No. of dead aster plants[b]				Relative root-knot index[c]			
	Soil treated with Larvacide		Soil not treated		Soil treated with Dowfume G		Soil treated with Dowfume W-15	
	Wet[d]	Dry[e]	Wet[d]	Dry[e]	Wet[d]	Dry[e]	Wet[d]	Dry[e]
1 day	0	4	10	11	2.2	2.9	0.0	2.2
3 days	5	11	10	11	2.3	2.3	0.0	3.2
6 days	11	11	10	11	2.6	2.8	0.8	2.9
Totals	16	26	30	33	7.1	8.0	0.8	8.3

[a] All test organisms were removed 14 days after the introduction of the fumigant.
[b] 12 dead plants represents no reduction in viability, and 0 dead plants represents complete kill, of the organism.
[c] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.
[d] The moisture level of the wet soil was maintained at 17 to 20 per cent.
[e] The moisture level of the dry soil was maintained at 7 to 10 per cent.

days after the introduction of the fumigant and tested for viability. This was to provide information on the retention of the fumigant in the soil in sufficient quantity to reduce viability of the test organisms. Data are given in tables 5 (effect of moisture) and 6 and 7 (effect of temperature) for Larvacide, Dowfume G, and Dowfume W-15. Data were not obtained for D–D, and *Sclerotium rolfsii* was not used in these tests.

Larvacide was effective in wet soil for a longer time than in the dry soil (Table 5). There was a sufficient amount remaining in the wet soil after 1 day to kill the Fusarium introduced at that time. It was slightly effective after 3 days in the wet soil but not in the dry soil, and was not effective after 6 days in either the wet or dry soil. Dowfume G was relatively ineffective after 1 day in either the wet or dry soil, although the root-knot

TABLE 6.—*Effect of temperature on the retention of Larvacide in the soil as indicated by the survival of Fusarium oxysporum f. callistephi introduced into the soil 1, 3, and 6 days after the introduction of the fumigant*[a]

Fungus added after:	Number of dead aster plants[b] at						Totals
	52°F.	53°F.	58°F.	72°F.	82°F.	98°F.	
1 day	0	1	2	1	0	0	4
3 days	2	4	2	2	2	4	16
6 days	4	3	4	3	4	4	22
Totals	6	8	8	6	6	8	42

[a] All test organisms were removed 14 days after the introduction of the fumigant.
[b] Four dead plants represents no reduction in viability; 0 dead plants represents complete kill of the Fusarium.

index was slightly less in wet than in dry soil. Dowfume W-15 was effective in the wet soil for a much longer time than in the dry soil. A sufficient amount of the fumigant was present after 3 days to give complete kill of *Heterodera marioni*, and almost complete kill occurred when the bags of nematode galls were buried in the soil 6 days after the introduction of the fumigant.

The effects of temperature on the retention of Larvacide are given in table 6, and for Dowfume G and Dowfume W-15 in table 7. Larvacide was still effective after 1 day regardless of temperature and was slightly effective after 3 days at temperatures of 52°, 58°, 72°, and 82° F., but not at 53° and 98°. Its ineffectiveness after 3 days at 98° was expected, but no satisfactory explanation for its ineffectiveness at 53° after 3 days can be given.

Dowfume G (Table 7) was not effective at 98° F. after 1 day but there was a slight effect at all the other temperatures even when the root-knot

TABLE 7.—*Effect of temperature on the retention of Dowfume G and Dowfume W-15 in the soil as indicated by the survival of Heterodera marioni introduced into the soil 1, 3, and 6 days after the introduction of the fumigant*[a]

Fungus added after:	Relative root-knot index[b] at						Totals
	61–70°F.	61–71°F.	64–72°F.	72–80°F.	80–86°F.	90–101°F.	
			Dowfume G				
1 day	2.3	2.5	2.8	1.8	2.0	4.0	15.4
3 days	2.0	2.3	2.3	2.0	2.0	4.0	14.6
6 days	2.8	2.3	2.5	2.3	2.5	4.0	16.4
Totals	7.1	7.1	7.6	6.1	6.5	12.0	46.4
			Dowfume W-15				
1 day	0.8	1.0	1.0	1.0	1.0	1.8	6.6
3 days	1.8	1.8	1.8	1.3	1.0	2.0	9.7
6 days	1.3	1.8	1.3	1.8	2.0	3.0	11.2
Totals	3.9	4.6	4.1	4.1	4.0	6.8	27.5

[a] All test organisms were removed 14 days after the introduction of the fumigant.
[b] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.

galls were buried in the soil 6 days after the introduction of the fumigant. The ethylene dichloride constituent of the Dowfume G may be responsible for the reduction in viability of *Heterodera marioni* after this long period. It has nematocidal properties as well as a much higher boiling point than methyl bromide (3) and would be expected to remain in the soil longer and be less affected by temperature than methyl bromide. Dowfume W-15 was most effective against the nematodes after 1 day at 52° F. and least effective at 98°. Fairly effective concentrations remained in the soil at temperatures of 52° to 58° for 6 days, but not at 98°.

DISCUSSION

Stark and Lear (12) have pointed out that there are limitations to greenhouse tests with soil fumigants. Such limitations are recognized in the tests reported herein. Nevertheless, it is believed that such tests are necessary for the proper evaluation of the effects of temperature and moisture on the efficacy of soil fumigants. Because of conflicting reports on the efficiency of soil fumigants over a wide range of temperatures, it is necessary that tests be conducted for determining the effects of temperature and moisture. This knowledge is particularly essential now that fall application of soil fumigants in northern areas is being advocated (1). If it can be shown that soil fumigants can be used effectively in the fall after crops have been harvested and when soil temperatures are low, many farmers would be willing to reclaim their soil by such treatment. When soil is treated in the fall the fumigant should be dissipated by spring so that crops could be sown early in the season.

The tests reported herein bear on this question, and, although far from adequate, they do indicate that fumigants may be effective at lower temperatures, although the action is slow. Larvacide without a water seal was surprisingly effective in these tests. Furthermore, it was retained for 1 to 3 days in wet, low-temperature soils in sufficient concentration to be toxic to *Fusarium oxysporum* f. *callistephi*. Many more tests of this type are needed, particularly for the low temperature ranges and for different soil types, before fumigation of low-temperature soils can be recommended.

SUMMARY

The efficacy of Larvacide, D–D, Dowfume G, and Dowfume W-15 against *Heterodera marioni* (Cornu) Goodey, *Fusarium oxysporum* f. *callistephi* (Beach) Snyder and Hansen, and *Sclerotium rolfsii* Sacc. at two soil moisture levels and six soil temperatures varying from 45° to 101° F., was investigated.

Larvacide was most rapidly effective against all three organisms at 98° F. It was only slightly effective against *Heterodera marioni* at the lower temperatures.

D-D was not effective against *Fusarium oxysporum* f. *callistephi* or *Sclerotium rolfsii*, but was effective against *Heterodera marioni*, causing complete kill at 82° or 98° F. after 1 day and at 62° or 72° after 3 days. *Heterodera marioni* was affected only slightly by D-D even after 3 days or more at 45° or 55° F.

Dowfume G showed no effect against *Fusarium oxysporum* f. *callistephi* and only slight effect against *Heterodera marioni*. There was a considerable reduction in viability of the sclerotia of *Sclerotium rolfsii* after 3 days at 72°, 82°, or 98° F. This may have been caused by the action of antagonistic organisms.

Dowfume W-15 was not effective against *Fusarium oxysporum* f. *callistephi* but was effective against *Sclerotium rolfsii* at 72°, 82°, or 98° F. after 6 days. It was most effective against *Heterodera marioni* at 98° after 1 day and after 3 days was effective at all temperatures.

In general, the fumigants were most effective in wet soils, and they were retained longest in the wet, low-temperature soils.

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THREE INTERSPECIFIC HYBRIDS OF TOBACCO

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Interspecific hybrids of *Nicotiana tabacum* L. with three other species of *Nicotiana* are recorded in this paper. They were attempted primarily for their possible resistance to downy mildew or blue mold (*Peronospora tabacina* Adam), blackshank (*Phytophthora parasitica* Dastur var. *nicotianae* Tucker), and root knot (*Heterodera marioni* Goodey). It is thought that they may be of interest (a) for the methods used and the types of hybrids produced, (b) for possible genetic, cytological, and chemical studies, and (c) for further breeding to introduce disease resistance and perhaps other desirable characteristics into various commercial types of tobacco.

The pistillate parent of each hybrid was of the commercial cigar-wrapper (shade) tobacco variety Rg, originated at this Station (8), and highly resistant to blackshank in the field in Florida. Without artificial shade, it commonly grows to a height of 8 feet, and produces about 26 leaves.

The staminate parents were of the species *Nicotiana debneyi* Domin., *N. repanda* Willd. *ex* Lehm., and *N. plumbaginifolia* Viv. The seeds were originally obtained from the Division of Tobacco, Medicinal, and Special Crops, United States Department of Agriculture. Resistance of these species to several diseases is reported in the literature available. *N. debneyi* is highly resistant to downy mildew (4), and also resistant to black root rot and wildfire (5). *N. repanda* is highly resistant to root knot (3, 5), and to black root rot and wildfire (5), and also resistant to blackshank (6). *N. plumbaginifolia* is highly resistant to downy mildew (4); it is also resistant to root knot, the nematodes from tomato entering the roots but developing only slightly and producing no eggs (2). In field tests at Quincy, Florida, *N. repanda* and *N. plumbaginifolia* were moderately resistant to blackshank.[1]

Several attempts to produce the hybrids described in this paper are reported in the literature. Kostoff, in a critical review published in 1941, described his sterile hybrids of *Nicotiana tabacum* and *N. debneyi* (9, p. 698), and reviewed a report by Pal and Nath on their sterile *N. tabacum* × *N. plumbaginifolia* hybrids (9, p. 656). No report of a fertile cross between *N. tabacum* and *N. repanda* has been found in the literature, but Foster (6) obtained seedlings of *N. repanda* (4-N) × *N. tabacum* (2-N) which failed to recover after transplanting. Clayton (4) reported successful crosses of *N. tabacum* with *N. debneyi* and *N. plumbaginifolia*.

The methods used in this work were conventional, except that the method of mixed pollination (1) was used in some attempts. A small amount of

[1] *N. megalosiphon* Huerk and Muell. is reported resistant to downy mildew (4) and to root knot (2, 3), and at Quincy, Florida, was moderately resistant to blackshank. Two unsuccessful attempts have been made at this Station to produce the hybrid, *N. tabacum* × *N. megalosiphon*, but Clayton (4) reported having made this cross.

self pollen was applied to the stigma along with a large amount of foreign pollen to insure the setting of the capsule, although no great difficulty was experienced without this precaution. All F_1 plants were examined for the purpose of detecting hybrids.

Each F_1 hybrid was transplanted into a 4-gallon jar and grown to maturity either in the greenhouse or outdoors. The identifying number was assigned when the first hybrid seeds were produced.

Nicotiana tabacum × *N. debneyi*

This cross was attempted once in 1940 without self pollen, with no success. It was attempted again in 1941 and 1942, using mixed pollination. The 1942 attempt (No. 798) yielded three seedlings which appeared to be hybrids. After transplanting, they grew slowly to heights of from $7\frac{1}{2}$ to $9\frac{1}{2}$ feet, produced from 41 to 54 small leaves, and bloomed from 2 to 4 months later than normal for the *Nicotiana tabacum* parent. One of them is shown in figure 1. All were nearly self-sterile, but set seed readily with pollen of *N. tabacum*. All produced fertile F_2 progeny, and were resistant to blackshank. No. 798–3 was of fairly good cigar-wrapper type.

The progenies of 798–1 and 798–3 had no appreciable resistance to downy mildew in the plant bed, but possessed some resistance to downy mildew leaf spotting in the field. This was apparently associated with a characteristic which they have in common with *Nicotiana debneyi;* that is, moisture (such as rainwater) spreads in a thin film over the surface of the leaves and hence evaporates quickly. Leaves of the Rg variety of tobacco collect large drops of water and remain wet for a considerably longer time. The quick-drying characteristic may be of value in connection with resistance to other leaf diseases, and possibly of agronomic value. No. 798–2, which did not have the quick-drying characteristic, was not tested for downy mildew resistance in the plant bed; it had no resistance in the field.

It is an interesting coincidence that Chileno Correntino, a tobacco variety introduced from Argentina (T. I. 57), moderately resistant to downy mildew (7), also has the quick-drying characteristic. This suggests the possibility that Chileno Correntino may have originated as a cross between *Nicotiana tabacum* and *N. debneyi.*

In crosses between tobacco varieties Rg and Chileno Correntino, the quick-drying characteristic appeared to be due to a single dominant factor.

Nicotiana tabacum × *N. repanda*

This cross was attempted three times without self pollen, with no success. The 1941 attempt (No. 784), using mixed pollination, yielded one seedling which appeared to be a hybrid. It grew rather slowly to a height of 6 feet, produced 48 small leaves, and bloomed 2 months later than normal for the *Nicotiana tabacum* parent. It produced a large crop of viable seed. The F_2 (Fig. 2) and succeeding generations were highly resistant to blackshank; some lines were moderately resistant to root knot. The leaves were thick and of no value as cigar wrappers.

Nicotiana tabacum × *N. plumbaginifolia*

Of four attempts made to accomplish this cross without self pollen, three yielded one hybrid plant each, all self fertile.

No. 853 (1945) grew normally in the greenhouse during the winter, and resembled the *Nicotiana tabacum* parent rather closely. The F_2 and later

FIG. 1 (*Left*). *N. tabacum* × *N. debneyi* hybrid, F_1 (No. 798).
FIG. 2 (*Right*). *N. tabacum* × *N. repanda* hybrid, F_2 (No. 784–1).

progeny were resistant to blackshank, and some F_4 lines appeared resistant to root knot in 1948.

No. 871 (1946) grew slowly, produced a moderate number of leaves, and bloomed 2 months later than normal for the *Nicotiana tabacum* parent. Plants of the F_2 generation in 1948 were large and coarse, and rather susceptible to blackshank, but were apparently resistant to root knot.

No. 870 (1946) grew very slowly, produced a large number of leaves, and bloomed 4 months later than normal for the *Nicotiana tabacum* parent.

Plants of the F_2 generation in 1948 had a large number of medium-size, thick leaves, and short internodes. All of the 1948 crop died of blackshank.

No. 870 in the F_2 generation closely resembled the White Honduras variety of tobacco in the small size of seeds and slow growth of seedlings, the appearance of the plants, and the extreme susceptibility to blackshank. Considered together with the root-knot resistance of both White Honduras (7) and *Nicotiana plumbaginifolia* (2), these similarities suggest the possibility that White Honduras originated as a cross between *N. tabacum* and *N. plumbaginifolia*.

In summary, this paper records the production of fertile hybrids between *Nicotiana tabacum* L., cigar-wrapper variety Rg, as the pistillate parent, and *N. debneyi*, *N. repanda*, and *N. plumbaginifolia*. All of the hybrids resembled the *N. tabacum* parent much more closely than they did the other parent. Their resistance to certain tobacco diseases and some other characteristics are recorded.

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DIFFERENTIATION OF TOBACCO AND TOMATO RINGSPOT VIRUSES BY CROSS IMMUNIZATION AND COMPLEMENT FIXATION[1]

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INTRODUCTION

Serological reactions and cross immunity tests have proved extremely useful for differentiating plant viruses. When used for this purpose, the results of the two methods have generally been in strict accord (1, 2, 3, 4, 5). However, in a few cases (5, 11, 12, 13) the relationships among plant viruses indicated by serological studies have not agreed with those indicated from cross immunity tests. Price (10) showed by cross immunity tests that tomato ringspot virus was distinct from tobacco ringspot virus. Chester (5), on the other hand, found by precipitin tests that these two viruses had similar antigenic structures.

In view of the seemingly contradictory evidence reported by Price and Chester, experiments were made to re-examine the serological and cross immunological relationships of the two viruses. As a further control on the validity of the methods, a new undescribed strain, here designated as the Pennsylvania strain of tobacco ringspot virus or more simply as Pennsylvania ringspot virus, was included in the experiments.

The methods chosen were the plant cross immunity and complement fixation tests. Although Chester (4) did not obtain a successful complement fixation test with tobacco ringspot or tomato ringspot viruses, nor has one been reported in the literature so far as can be determined by the authors, the complement fixation technique used here proved both sensitive and specific.

MATERIALS AND METHODS

The plant viruses used were as follows:

(a) Tobacco ringspot virus, *Annulus tabaci* H. var. *virginiensis* H., has been described in detail by Fromme, Wingard, and Priode (7), and by Wingard (17). It was obtained originally from Dr. Wingard.

(b) Tomato ringspot virus, *Annulus zonatus* H., was originally isolated and described by Price (10), who designated it tobacco ringspot virus No. 2. The name was later changed and reported in Holmes' (9) handbook.

(c) The Pennsylvania strain of tobacco ringspot virus, *Annulus tabaci* H. var. *Pennsylvanicum* n.v., was first seen on flowering tobacco plants in the horticultural gardens at Pennsylvania State College. The symptoms and behavior of this virus on tobacco plants are similar to those of the type strain. However, in this host it differs from the type in being somewhat

[1] Based on a thesis submitted by the senior author in partial fulfillment of the requirements for the Master of Science degree.

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more infectious, in showing somewhat more rapid movement, and in usually producing slightly larger zonate necrotic lesions. Evidence for close relationship between the Pennsylvania and type strains will be discussed under the experimental section.

Stock cultures of the viruses were maintained in Turkish tobacco plants in a greenhouse that was fumigated at frequent intervals to control insects.

Methods of Inoculation

Except where otherwise noted, the rubbing method of inoculation was used. Cheesecloth pads were moistened with a virus preparation and gently rubbed over the surface of test leaves. For mass transfers, an infected leaf was wrapped in a square of cheesecloth and macerated by pounding with a pot label, and the resulting substance was rubbed over the leaves. Immediately after inoculation the leaves were sprinkled with water. In special cases inoculations were carried out by cleft grafting.

Host Plants

Turkish type tobacco (*Nicotiana tabacum* L.), Holmes' (8) necrotic-type hybrid (*N. digluta* Clausen and Goodspeed × *N. tabacum* L.), and Bountiful bean (*Phaseolus vulgaris* L.) were used as host plants. All plants were grown in composted soil in 4-inch pots in the greenhouse. Symptoms produced in Turkish type and necrotic-type tobacco plants were indistinguishable. Bountiful bean plants infected with either the type or Pennsylvania strain of tobacco ringspot virus developed necrotic, ringlike, local lesions and systemic necrosis within five days. The viruses soon thereafter involved the terminal bud and resulted in death of the plant. Bountiful bean plants infected with tomato ringspot virus showed necrotic, ringlike, local lesions similar to those produced by tobacco ringspot virus. They either failed to show evidence of systemic infection or developed zonate systemic lesions in from seven to ten days after inoculation. Terminal buds were killed in only a few instances. The variability in movement of tomato ringspot virus within bean plants is comparable to that observed in Turkish tobacco plants (10).

Cross Immunity Tests

Turkish tobacco and necrotic-type tobacco plants were inoculated when they had reached a height of about six inches. Such plants were held on a greenhouse bench until they had recovered and were then used as a source for tip cuttings. Cuttings were rooted in a mixture of moist peat moss and sand and then transferred to composted soil in 4-inch pots. When they had reached a height of about six to eight inches, they were used as test plants in all cross protection experiments.

Complement Fixation Tests

Since viruses used in complement fixation tests were not completely purified, antigens used to immunize rabbits were prepared from Turkish

tobacco plants while those used to react with antisera were prepared from Bountiful bean plants. This was done on the assumption that the antigenic constituents of normal tobacco plants were serologically distinct from those of normal bean plants, an assumption which later was shown to be correct.

Except where otherwise mentioned, the procedure for preparing antigens was as follows. Leaves inoculated ten days earlier and showing numerous necrotic spots were surface-sterilized for ten minutes in a 1:10,000 $HgCl_2$ solution. The leaves were then passed through four sterile saline washes and crushed aseptically under a hood in a mortar containing sterile alundum. The crushed tissue was spun in an angle centrifuge for thirty minutes at approximately 2500 rpm. A turbid green supernatant extract was obtained, poured off, and frozen at $-20°$ C. for 24 to 36 hr. The fluid extract was thawed and spun again for 30 min. at 2500 rpm. Following this centrifugation, a clear, straw-colored supernatant fluid was obtained. The antigen in this form was pooled, thoroughly agitated, and dispensed in sterile serum bottles in 5-ml. lots. The antigens were then labeled and stored at $-20°$ C. until needed.

Preparation of Antisera. Approximately 25 ml. of blood was taken aseptically from each of four rabbits by means of cardiac punctures. Each of the four rabbits was then injected with antigen preparations containing normal tobacco sap, tobacco ringspot virus, Pennsylvania ringspot virus, and tomato ringspot virus, respectively. A total of seven intraperitoneal injections in about 4-ml. amounts were administered to each rabbit. One week after the last injection, approximately 25 ml. of blood was taken aseptically from each rabbit by cardiac puncture.

Normal sera and immune sera were separated aseptically from blood samples in the following manner: Each sample of blood was allowed to clot for one hour at room temperature, then rimmed and stored overnight at 10° C. The blood was then spun in an angle centrifuge, and the supernatant serum removed. Each serum was dispensed in 1-ml. lots, labeled, and stored at $-20°$ C. until needed. All sera used in antigen and antiserum titrations were inactivated at 56° C. for 30 min.

Preliminary antigen titrations indicated that the tomato ringspot antiserum contained no antibodies demonstrable by the complement fixation tests used. In view of this finding, two additional 7-ml. injections were administered over a two-week period. The rabbit was bled seven days after the final injection, and the antiserum was separated as previously described. As will be seen later, not even the two additional injections of tomato ringspot antigen was sufficient to raise the demonstrable antibody titer to a significant level. Consequently the virus was concentrated about twenty-five times by high speed centrifugation and the concentrate injected into the same rabbit. These injections proved effective in inducing specific complement-fixing antibodies.

The complement fixation technique used in these experiments has been

described in detail by Wertman (16). Commercial Antisheep Hemolysin Glycerinized obtained from Sharp and Dohme and sheep erythrocytes obtained from Brown Laboratory, Topeka, Kansas, were used in preparing the hemolytic system. Complement was obtained from Sharp and Dohme under the trade name Lyovac Complement.

RESULTS OF CROSS IMMUNITY TESTS

Cross immunity tests were performed to determine whether or not tobacco plants that had recovered from any one of the three ringspot virus diseases would be immune from either of the other two ringspot diseases. For this purpose, one set of cross protection tests was carried out on Turkish tobacco plants and another set was carried out on necrotic-type hybrid plants.

Sets of cuttings each containing three or more plants grown from healthy Turkish and necrotic-type tobacco plants and from plants that had recovered from tobacco ringspot, Pennsylvania ringspot, and tomato

TABLE 1.—*Summary of cross protection tests with recovered plants of Turkish and necrotic-type tobacco*

Virus used for inoculation	Plant recovered from			
	Tobacco ringspot	Pennsylvania ringspot	Tomato ringspot	Controls
Tobacco ringspot	0	0	+	+
Pennsylvania ringspot	0	0	+	+
Tomato ringspot	+	+	0	+

0 indicates complete protection.
+ indicates no protection.

ringspot viruses, respectively, were challenged in all possible combinations with the three viruses. The challenge inoculation was made by rubbing in one set of experiments and by cleft grafting in another.

Results of these experiments were identical and are summarized in table 1. Plants that had recovered from tobacco ringspot virus developed no lesions when reinoculated with the same virus or with Pennsylvania ringspot virus. Similarly, plants that had recovered from Pennsylvania ringspot virus showed no lesions when reinoculated with the same virus or with tobacco ringspot virus. On the other hand, plants that had recovered from either Pennsylvania or tobacco ringspot virus developed lesions when reinoculated with tomato ringspot virus. Plants that had recovered from tomato ringspot virus showed no lesions when reinoculated with the same virus, but developed lesions when inoculated with either the Pennsylvania or tobacco ringspot viruses.

Because of the complete protection between tobacco ringspot and Pennsylvania ringspot virus, it can be concluded that these viruses are closely related strains belonging to the same virus group. The failure of these

two virus strains to protect against tomato ringspot virus, together with the failure of tomato ringspot virus to protect against the two strains of tobacco ringspot virus, indicates that these viruses are not closely related strains and belong in separate virus groups.

RESULTS OF SEROLOGICAL STUDIES

Activity and Sterility Tests

Activity tests were conducted with each virus antigen to determine the extent of active virus present in the preparation. Dilution end points were determined by rubbing dilutions of $10^{-0.5}$, $10^{-1.0}$, $10^{-1.5}$, $10^{-2.0}$, and $10^{-2.5}$ of the antigen preparation over test leaves of Turkish tobacco plants. Each dilution was tested on five leaves of each of four plants and the infections were recorded 15 days later. The dilution end point of antigens prepared from both Turkish tobacco and Bountiful bean plants was $10^{-2.0}$ for both tobacco and Pennsylvania ringspot virus, and $10^{-1.0}$ or less for tomato ringspot virus.

Bacterial plate counts on nutrient agar were made with each antigen to determine whether or not bacterial contamination would be sufficient to interfere with serological tests. Three ml. of a representative sample were plated in 10 ml. of nutrient agar and incubated at 37° C. Counts observed after 48 hr. incubation varied from none to five organisms per ml. The data indicate that the number of bacteria present in the prepared antigens was too small to cause interference with the specific complement fixation tests.

Complement Fixation Tests

Experiments were carried out to determine whether the serological relationships between the three viruses would agree with the immunological relationships previously found. The plan of the experiments was to compare the three viruses on the basis of their antiserum dilution end-point for complete fixation of complement in the presence of homologous and heterologous antigens. Before this could be done it was necessary to standardize the amount of hemolysin to be used in all subsequent tests, to standardize the amount of complement to be used in antigen titrations, and to standardize the amounts of both complement and antigen to be used in serum titrations.

Hemolysin titration. Titration of hemolysin was performed by adding 0.25 ml. of 3 per cent suspension of washed sheep erythrocytes to 0.25 ml. of varying dilutions of hemolysin. The tubes were allowed to stand for 10 min. at room temperature and then 0.50 ml. of a 1 : 30 dilution of complement and 0.50 ml. of fresh saline (0.85 per cent) were added. The tubes were incubated at 37° C. for 30 min. in a water bath, and read. The tube with the highest dilution of hemolysin showing complete hemolysis of sheep red blood cells was taken as one MHD (minimal hemolytic dose) of hemolysin. Three MHD of hemolysin were used in complement, antigen, and

serum titrations. This system is referred to here and in later sections as sensitized red blood cells.

Complement titrations. Complement titrations were conducted before each antigen titration and before serum titrations. Titrations were carried out with varying quantities, *i.e.* 0.08, 0.10, 0.12 . . . 0.26 ml. of a 1:30 dilution of complement. The titration was made in the presence of 0.25 ml. of diluted antigen. The dilution of antigen used in complement titration prior to antigen titrations was 1:10. Thereafter the dilution of antigen used in complement titrations was comparable to the amount used in subsequent serum tests. Table 2 shows the amount of materials used in complement titration. Final readings were taken after incubation at 37° C. for 30 min. The first tube showing complete hemolysis of the sensitized red blood cells was taken as the exact unit and the tube with the next highest amount of complement as a full unit. Two full units contained in 0.50 ml. of fresh saline (0.85 per cent) were used in the antigen titrations

TABLE 2.—*Milliliters of various materials used in titration of complement*

Material	Tube number									
	1	2	3	4	5	6	7	8	9	10
	Incubated at 37° C. for 30 min.									
Complement (1:30)	0.08	0.10	0.12	0.14	0.16	0.18	0.20	0.22	0.24	0.26
Saline (0.85 per cent)	0.67	0.65	0.63	0.61	0.59	0.57	0.55	0.53	0.51	0.49
Antigen[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sensitized R.B.C.	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[a] Dilution of 1:10 was used in titration prior to antigen titration. Dilution containing two antigenic units was used in titration prior to serum titration.

and in the final titration of sera. All dilutions in these and subsequent tests were made in fresh saline.

Antigen titration. Each antigen was titrated to find the highest dilution of antigen which, when mixed with homologous antiserum, would fix complement over a range of antiserum dilutions. This range should be wide enough to permit comparisons between the reactions of antigen with homologous and heterologous antisera in serum titrations. This dilution of antigen was designated as one antigenic unit. Two antigenic units were used in subsequent serum titrations.

The antigen titration was carried out by mixing 0.25 ml. of twofold serial dilutions of antigen with equal volumes of twofold serial dilutions of homologous antiserum in the presence of two units (0.50 ml.) of previously titrated complement. Normal serum controls were included as well as the usual antigen and antiserum controls. Twofold serial dilutions of normal bean antigen were mixed with the antiserum and normal serum and served as additional controls. The purpose of this was to determine whether or not the serum of rabbits immunized with virus extracts of tobacco plants would fix complement in the presence of extracts of normal bean plants. As will be seen later, no significant cross reaction occurred.

At the same time a second complement titration, in the absence of antiserum and in the presence of each antigen, was carried out as a control. Varying amounts of diluted complement, *i.e.*, 0.10, 0.20, 0.25 (one full unit), 0.30, 0.35, 0.40, and 0.50 (two full units) ml. were mixed with 0.25 ml. of a 1:10 dilution of antigen. The dilution of complement used was equal to that found in the preliminary titrations. Fresh saline was added to all tubes in amounts necessary to bring the final volume to 1.50 ml. After a primary incubation for 18 hr. at 4°–8° C., 0.5 cc. of sensitized red blood cells was added to each tube which was then incubated for 30 min. at 37° C. and read. The purpose of this second complement titration was twofold: (1) to determine whether or not there had been any inactivation of complement during the primary incubation period, and (2) to check on the validity of the preliminary complement titration. Thus it served to demonstrate that all fixation of complement was due to antigen-antibody reaction.

The mixtures were incubated at 4°–8° C. for 15 to 18 hr. Following this, 0.50 ml. of sensitized red blood cells was added to each tube. The tubes were shaken, incubated in a water bath at 37° C. for 30 min., and read. The antigenic unit was recorded in terms of the dilution originally added to the tubes. All antigen titrations were repeated several times, each repetition confirming the original findings.

Results obtained from titrations showed that a 1:15 dilution of both tobacco and Pennsylvania ringspot viruses contained two units of antigen. However, the titration of tomato ringspot virus indicated that complement-fixing antibodies were either absent from the antiserum or present in such low concentrations as to make subsequent serum titrations insignificant. For this reason, it was necessary to concentrate the virus by high speed centrifugation and to make additional injections with the concentrated material.

Serum titration. Tests were conducted to determine the dilution endpoint for complete complement fixation of antiserum for each of the three viruses in the presence of two units of homologous and heterologous virus antigens. In these tests, 0.25 ml. of solution containing two units of antigen was mixed with 0.25 ml. of twofold serial dilutions of antiserum. All possible combinations of antigens and antisera were made. The dilution of antiserum ranged from 1:12 to 1:768.

Three normal rabbit serum controls were included, as well as the usual antigen and antiserum controls. Dilutions of normal tobacco antiserum were mixed with the three antigens and served as additional controls. The purpose was to determine whether or not the serum of a rabbit immunized with normal tobacco sap would fix complement in the presence of virus antigens prepared from bean plants. Two full units of complement in 0.50 ml. were added to all mixtures of antigen and serum.

The mixtures of serum, antigen, and complement were incubated at 8° C. for 16 hr. Following this, 0.50 ml. of sensitized red blood cells was

added. The mixtures were then incubated in a water bath at 37° C. for 30 min. and final readings were made. End points were estimated on the basis of the last tube showing complete fixation of complement and the titer recorded as the dilution of serum originally added to that tube. As in the case of the antigen titrations, a second complement titration was made but in this case two units of each antigen were used. Again this served as a check on the preliminary complement titration and demonstrated that all fixation of complement was due to antigen-antibody reaction.

The data are summarized in table 3. The conclusions to be derived from these results are as follows:

(1) Normal antigenic constituents of Turkish tobacco plants differ from those of Bountiful bean plants since antibodies formed against nor-

TABLE 3.—*Summary of complement fixation tests*

Sera	Antigens			
	Normal bean sap 1:6	Tobacco ringspot virus[a] 1:15	Pennsylvania ringspot virus[a] 1:15	Tomato ringspot virus[a] 1:12
Normal rabbit No. 1	0[b]	0	0	0
do No. 2	0	0	0	0
do No. 3	0	0	0	0
Tobacco sap	0	0	0	0
Tobacco ringspot virus	0	1:384[c]	1:384	0
Pennsylvania ringspot virus	0	1:384	1:384	0
Tomato ringspot virus[d]	0	0	0	1:48

[a] Two antigenic units, in all cases prepared from infected bean plants.
[b] No fixation of complement.
[c] Dilution of antiserum showing complete complement fixation.
[d] Serum prepared by injection of virus concentrated 25 times by high-speed centrifugation of infected tobacco plants.

mal tobacco sap did not react with either normal bean sap or with virus antigens prepared from bean plants.

(2) Antigen preparations of tobacco and Pennsylvania ringspot viruses induce the formation of demonstrable complement-fixing antibodies in rabbits.

(3) Because antisera prepared against tobacco and Pennsylvania ringspot viruses completely fixed complement to almost equal dilution endpoints with either virus antigen, it can be concluded that these viruses have similar antigenic structures. This would indicate that the two viruses are closely related strains and belong in the same virus group. The serological relationship is in complete accord with that indicated by cross protection tests.

Tests with Centrifuged Tomato Ringspot Virus

Preliminary results obtained with tomato ringspot virus suggested that the preparations used contained insufficient virus to induce antibody formation. Tests were therefore carried out with a tomato ringspot virus prepara-

tion that had been concentrated about 25 times by high-speed centrifugation.[2] Leaves from 69 infected tobacco plants were harvested and crushed with a mortar and pestle. The sap was extracted, centrifuged at 3,000 r.p.m. for 15 min., then frozen overnight at – 10° C., thawed and again centrifuged at low speed. Two hundred forty cc. of the clear green fluid supernatant was spun at 33,560 r.p.m. for 1½ hr. The supernatant from the centrifugation was discarded. The pellet was suspended in 10 cc. of sterile 0.85 per cent saline and centrifuged at 5,000 r.p.m. for 10 min. The clear, slightly greenish supernatant was used as an immunizing antigen. It produced numerous local lesions on tobacco plants at a dilution of $10^{-1.5}$ and a few lesions at $10^{-2.0}$.

The concentrated preparation of tomato ringspot virus was used to inject the same rabbit used previously for tomato ringspot virus immunization trials. The rabbit was injected intraperitoneally with 2 cc. of the concentrated antigen and 6 days later with 5 cc. of the same antigen. It was bled 8 days after the final injection. Complement fixation tests were carried out in the manner previously described, with the immune serum mixed with an antigen prepared from infected bean plants in the usual manner.

The data (Table 3) show that tomato ringspot virus, when sufficiently concentrated, is capable of inducing specific complement-fixing antibodies in rabbits. The failure of tomato ringspot virus antiserum to react with either the type or Pennsylvania strains of tobacco ringspot virus, together with the failure of tomato ringspot virus to react with antiserum for either strain of tobacco ringspot virus, strongly suggests that these two viruses are serologically unrelated.

DISCUSSION

The studies reported in this paper demonstrate that viruses of the tobacco and tomato ringspot groups induce the formation of demonstrable complement-fixing antibodies when injected into rabbits. The complement fixation technique used here proved sensitive and specific for both viruses. Chester (4) was unable to obtain a successful complement fixation test with tobacco ringspot virus, among others, finding that "In order for the reaction to be error-free, the immune serum and the virus extract must each be diluted to about 1 : 20. This double dilution lies so far beyond the point of precipitation with these viruses that even a complement fixation reaction is not obtained."

Data reported here show that a 1 : 15 dilution of tobacco ringspot virus completely fixed two units of complement in the presence of homologous antiserum diluted as high as 1 : 384. Likewise, a 1 : 12 dilution of tomato ringspot virus completely fixed two units of complement in the presence of homologous antiserum diluted 1 : 48. It is clear from this that the comple-

[2] The writers are indebted to Mr. T. Edward Cartwright, Jr., for carrying out the centrifugation.

ment fixation technique used by Chester was not nearly so sensitive as the technique described in this paper. This may be due to the different primary incubation periods used in the two techniques. Chester's primary incubation period for serum, virus extract, and complement was 1 hr. at 37° C. The primary incubation period used in these experiments was 15 and 18 hr. at 4° C.

The greater sensitivity of the technique using cold primary incubation is due to a more complete antigen-antibody reaction, for it was shown by second complement titration that two full units of complement remained after the primary incubation. This eliminated the possibility of some complement being inactivated during the incubation period, leaving only minimal amounts to be fixed.

Wertman (15) reported the effect of primary incubation on normal egg antigens when titrated against syphilitic serum. Three different periods of incubation were employed, *i.e.*, 1 hr. at 37.5° C., 4 hr. at 4° to 8° C., and 18 hr. at 4° to 8° C. He found that the greater fixation occurred when the 18-hr. incubation in the cold was utilized. Damon and Johnson (6) also reported greater sensitivity with icebox primary incubation. They concluded that the greater sensitivity of the icebox technique was especially important in testing sera having minimal concentration of antibodies.

Chester's (4) complement fixation technique proved inadequate not only for tobacco ringspot virus, but also for the viruses of cucumber mosaic, potato veinbanding, and potato aucuba mosaic. This failure, together with the fact that twenty-one other viruses and virus strains have failed to respond to any sort of serological reaction (5), indicates that there is a decided need for sensitive serological techniques in plant virus research. Further studies may show that the sensitive complement fixation technique described in this paper will prove successful for determining the antigenic relationships of some of those other viruses, and thereby be of further use in plant virus classification.

The symptomatology and general characteristics of the Pennsylvania strain of tobacco ringspot virus suggest its close relationship to the type strain of tobacco ringspot virus. This relationship was definitely borne out by the serological and immunological data obtained. Therefore, it can be concluded that the Pennsylvania strain of tobacco ringspot virus belongs in the tobacco ringspot group of viruses.

Tomato ringspot virus differs from viruses of the tobacco ringspot group in host range and symptomatology (10). Immunological results reported here show that these two groups of viruses are not closely related. They confirm those of previous investigations (10, 14). Direct serological evidence concerning this relationship was obtained with antiserum produced by injection of a rabbit with a tomato ringspot virus preparation concentrated by ultracentrifugation. This evidence contradicts that of Chester (5) who obtained a cross-precipitin reaction between tobacco ringspot and tomato ringspot virus. It is generally agreed that the complement fixation

reaction is not only more sensitive, but more reliable than the precipitin reaction. We therefore conclude on the basis of our serological and immunological results and of previous immunological results (10) that tobacco ringspot and tomato ringspot viruses are not closely related.

SUMMARY

Tobacco ringspot virus and the heretofore undescribed Pennsylvania strain of tobacco ringspot virus elicit the formation of specific complement-fixing antibodies in the serum of rabbits. These antibodies can be demonstrated by the complement fixation technique described in this paper. Tomato ringspot virus likewise elicits the formation of specific complement-fixing antibodies when the virus in sap of diseased plants is concentrated at least twenty-five times by high speed centrifugation.

Dilutions of antiserum of tobacco and Pennsylvania ringspot viruses completely fix complement almost to the same dilution end-point with either the homologous or heterologous antigen. This indicates that the two viruses have similar antigenic structure and should be considered as strains belonging to the same virus group.

Tomato ringspot virus antiserum failed to react with either the type or Pennsylvania strain of tobacco ringspot virus. Conversely, antisera for neither the type nor Pennsylvania strain of tobacco ringspot virus reacted with tomato ringspot virus antigen. This result suggests that tobacco and tomato ringspot viruses are serologically unrelated.

Results of cross immunity tests with tobacco and Pennsylvania ringspot viruses were in accord with the serological results. Each virus protected tobacco plants completely against the other.

Results of cross immunity tests with tobacco and tomato ringspot viruses were likewise in accord with serological results. Neither virus protected tobacco plants against the other. It is concluded that the tomato and tobacco ringspot virus groups are immunologically and serologically distinct.

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NATURE OF SUSCEPTIBILITY TO HELMINTHOSPORIUM VICTORIAE AND RESISTANCE TO PUCCINIA CORONATA IN VICTORIA OATS[1]

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INTRODUCTION

The oat variety Victoria (C.I. 2401) of *Avena byzantina* C. Koch., introduced from Uruguay by the United States Department of Agriculture in 1927, has been used extensively in breeding varieties resistant to crown rust, *Puccinia coronata* Corda. Selections from crosses with Victoria were found to be hypersensitive to the prevalent races of *P. coronata* so that cells immediately surrounding the infection loci collapse before the fungus can produce uredospores (Fig. 1). This type of resistance is so effective that Victoria has been used to develop the outstanding rust-resistant varieties Tama, Boone, Control, Vicland, Cedar, Vikota, Ventura, Osage, Neosho, Overland, Victorgrain, Lectoria and Lega. It has been estimated that 75 per cent of the oat acreage in 1945 in the major oat-growing sections of the United States was seeded to selections from crosses in which Victoria was one of the parents.

The popularity of these varieties is jeopardized by the widespread development on them of Helminthosporium blight caused by *Helminthosporium victoriae* Meehan and Murphy (2, 3), first described in 1946. Within the short span of two years it has been estimated that the acreage of the above varieties was reduced by about 20 per cent, and the reduction would have been much greater had seed of suitable substitute varieties been available.

Helminthosporium victoriae is regarded as highly specialized, since it has not caused injury to varieties other than those possessing the "Victoria type" of resistance to crown rust. Meehan and Murphy (2, 3) have reported that the secondary striping effect (Fig. 1) from *H. victoriae* is due to a toxin. The linkage between susceptibility to *H. victoriae* and resistance to *Puccinia coronata* is complete according to Murphy and

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Meehan (4). The question thus arises as to whether these two reactions are dependent upon the same mechanism.

It was considered desirable, therefore, to determine whether the two fungi produced toxins which would induce similar injury to plants possessing hypersensitiveness to certain races of *Puccinia coronata* and complete susceptibility to *Helminthosporium victoriae*. The results of several experiments with these toxins are reported in this paper.

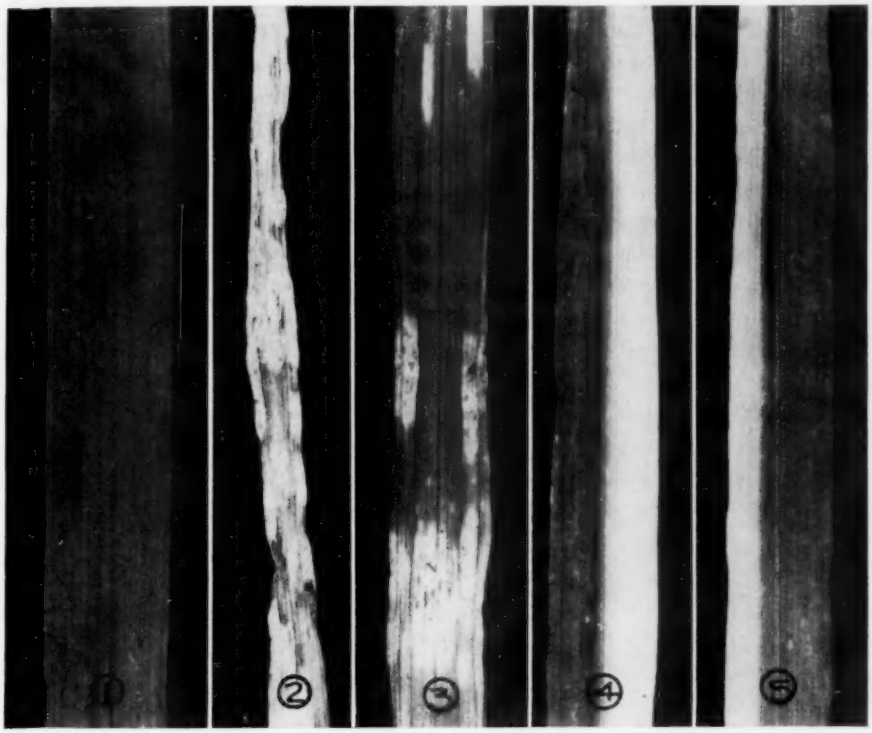

FIG. 1. Leaves of Victoria oats: 1. Normal. 2, 3. Necrosis resulting from infection with *Puccinia coronata*. 4, 5. Striping resulting from *Helminthosporium victoriae*.

EXPERIMENTAL TECHNIQUES

At least two contrasting varieties of oats were used in each experiment. In addition to a crown rust-hypersensitive and Helminthosporium blight-susceptible variety (Victoria, Vicland, or Tama), a variety resistant to *Helminthosporium victoriae* of Bond parentage (Bond or Clinton) was used. In two tests a series of varieties was used for comparative purposes.

The extracts containing the toxin of *Helminthosporium victoriae* were obtained from pure culture of isolate H96 (1) and from greenhouse plants inoculated with this culture. The cultures were grown on oatmeal agar in Petri plates at room temperature (approx. 80° F.) for at least 10 days or until the agar was completely covered with the developing fungus. The

mycelium from one Petri plate was scraped from the agar surface and macerated in 50 ml. distilled water in a Waring Blendor. For most experiments this mixture was steamed for 5 min. to kill the viable fungus and to facilitate release of the toxins. The concentrate was passed twice through Berkefeld filters to remove solid particles. The cleared extract, designated as the "basic concentrate," was then diluted with distilled water for testing.

In order to obtain comparable extracts, only seedlings having extensive basal infection were collected. The extracts were obtained either by subjecting the plants to 8,000 lb. pressure per sq. in. or by chopping them in a Waring Blendor with distilled water to liberate any toxins present. In either case the extract was steamed first and then passed through a Berkefeld filter. When difficulty was encountered in filtering, the solids were centrifuged out before filtering.

Toxins from crown rust were obtained by extracting plants infected with *Puccinia coronata* race 45. Infected leaves were harvested about 7 to 10 days after inoculation. At this time Victoria had not developed the pronounced necrotic flecks shown in figure 1, but Bond and other susceptible varieties had begun to produce sporulating pustules. Approximately 100 gm. plant material were diluted with 200 ml. distilled water in preparing the basic plant concentrate.

The effect on normal oat plants of the extracts from pure culture of *Helminthosporium victoriae* and also from infected plants was determined by several different methods. In preliminary tests the bloom was removed from the leaves of young oat plants, growing in pots, by passing them gently between moist fingers. Plants were sprayed with diluted extracts from various sources, placed in a moist chamber for about 8 hr. to retard drying and to permit maximum penetration, and then returned to the greenhouse. Observations on color, wilting, and necrosis were made several days later.

In other tests culms 4 to 18 in. long were cut at the ground line, re-cut under water, and inserted into the extracts. Usually the basic concentrate had been diluted with distilled water to several concentrations and about 20 ml. of each placed in a vial. A uniform number of cuttings ranging from 2 to 6 were placed in each vial in the different experiments. Final observations were made on wilting and necrosis within 4 days. In experiments where transpiration rates were measured, the vials were sealed with paraffin and weighed periodically.

The effect of the extracts on seedling development was determined by growing plants in soil extract agar and steamed or nonsteamed sand. The agar was prepared by a method employed by Dr. W. J. Hooker of the Iowa Agricultural Experiment Station, who has successfully used it in his studies with *Actinomyces scabies* (Thax.) Güssow. A 25-gm. sample of soil was added to a liter of tap water, allowed to stand for 24 hr., and decanted. The decanted liquid was made into 2 per cent agar medium,

sterilized and poured into 100-ml. beakers containing various concentrations of extracts. Seeds known to be free of disease organisms were dehulled, surface-sterilized in 10 per cent Clorox, and placed on the hardened agar. The beakers were covered with sterile Petri plates and kept at room temperature. The covers were removed when the growing seedlings began to be suppressed by them. Water was added as required after the seedlings began to develop. Although the method was not strictly aseptic, very few colonies of bacteria or fungi were observed. Similar methods were used with steamed and nonsteamed sand with due precautions against water-logging.

The treatments in the different experiments were arranged in randomized blocks whenever possible and replicated at least twice. The data for all major experiments were confirmed by repeated trials.

ABSORPTION OF EXTRACTS THROUGH LEAVES

Seedlings of Victoria and Bond that had attained a height of about 5 in. in 2-in. pots were de-waxed and sprayed with basic extracts from the culture of *Helminthosporium victoriae*, or from plants infected with either *H. victoriae* or *Puccinia coronata*. Suitable controls of each variety sprayed with water and healthy plant extract were included for comparison. The plants were placed in partial vacuum to encourage absorption of the spray deposit before they were placed in the humidity cabinet for slow drying.

All Bond plants remained normal during the 10-day period of observation irrespective of treatment. Severe injury was observed within 12 hr. on Victoria plants sprayed with extract from cultures of *Helminthosporium victoriae*. Necrotic lesions were numerous and during the first few days the leaves on severely affected plants dried. The final effect was very similar to the effect of a severe infection of crown rust on Victoria seedlings. Similar but less intense injury was observed on seedlings sprayed with extracts from plants infected with *H. victoriae*.

None of the injured plants died, as would have been expected if viable inoculum had been sprayed upon them. It was concluded, therefore, that the injury was due to a toxic substance or substances specific in effect on Victoria, as reported by Meehan and Murphy (3).

There was insufficient injury in this preliminary experiment to detect varietal difference in reaction to the extracts from plants infected with *Puccinia coronata*.

ABSORPTION OF EXTRACTS BY CUTTINGS

In order to obtain greater absorption of extract, a number of seedlings were cut and placed directly in portions of extracts. The plants were scored by visual observation into six classes, ranging from uninjured (Class 0) to complete yellowing, bronzing, and wilting (Class 5). Data on the effects of the various extracts are in table 1. They confirm obser-

vations in the preceding test except that the effects were more pronounced. Wilting of both Victoria and Bond cuttings was induced by the extracts from *Helminthosporium victoriae,* while characteristic streaking of leaves occurred only in Victoria cuttings. The presence of a toxic substance from *H. victoriae* in culture and from infected plants was fully confirmed. In addition, evidence was obtained of a mild toxic material from plants infected with *Puccinia coronata.* This material, as well as that obtained from plants infected with H. *victoriae,* also was specific to Victoria. No toxic effects on Bond were observed.

A second test was made with larger cuttings (about 18 in. tall) from Bond and Victoria. Duplicate plants of each variety were placed in 1 and 5 per cent concentrations of basic *Helminthosporium victoriae* extract, in extracts of *Puccinia coronata*-infected Bond and Victoria plants and of *H. victoriae*-infected Victoria plants. Results were approximately the same as those in the preceding experiment. The Bond variety was un-

TABLE 1.—*Effect of extracts from diseased and healthy oat plants and Helminthosporium victoriae on cuttings of Victoria and Bond oats*

Source of extract		Degree of damage[a] to cuttings	
Medium	Pathogen	Victoria	Bond
Victoria	None	1.3	1.3
Bond	None	2.0	1.5
Victoria	*P. coronata*	2.5	0.8
Bond	*P. coronata*	2.5	1.0
Victoria	*H. victoriae*	4.0	1.0
Culture	*H. victoriae*	5.0	2.0
Water	None	0.0	0.0

[a] Numerical rating: 0, uninjured; and 5, completely wilted and yellow.

affected except for slight wilting when placed in the more concentrated extracts (Fig. 2). The Victoria cuttings, on the other hand, were completely wilted and etiolated by *H. victoriae* extracts and were wilted appreciably by extract from *P. coronata*-infected plants. Victoria cuttings in water were unaffected, while cuttings in extract from healthy plants were only moderately affected.

In a third test, cuttings from 13 commercially important varieties possessing the hypersensitive type of resistance to *Puccinia coronata* and susceptibility to *Helminthosporium victoriae* of Victoria were compared with three *H. victoriae*-resistant varieties for reaction to extracts from diseased and healthy oat leaves. Cuttings from these varieties were also placed in water and a one per cent extract from *H. victoriae* for comparison. The cuttings were scored on a visual basis as described previously (Table 2).

Extracts from *Helminthosporium victoriae* and from leaves infected with this pathogen were specifically toxic to the cuttings of the 13 *H. victoriae*-susceptible varieties. They were scored 4.1 in *H. victoriae* extract and 4.8 in the extract from the diseased plants. The cuttings in water

were unaffected (Score 0) while those in the healthy plant extract averaged 2.2. The *H. victoriae*-resistant varieties had no symptoms of toxicity beyond that obtained from the healthy plant extract.

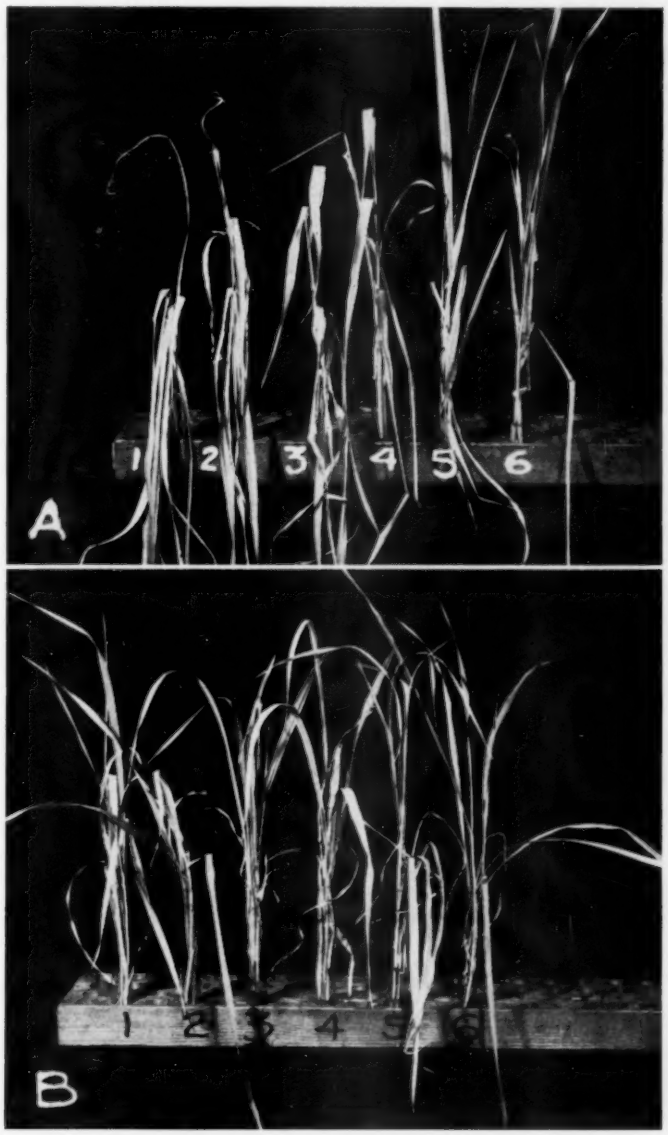

FIG. 2. (A) Victoria and (B) Bond oat cuttings after 48 hours in extract solutions: 1. A 5 per cent dilution of *Helminthosporium victoriae* culture. 2. A 1 per cent dilution of *H. victoriae* culture. 3. *H. victoriae*-infected plants. 4. *Puccinia coronata*-infected plants. 5. Healthy plants. 6. Water.

The toxicity patterns with extracts from *Puccinia coronata*-infected and healthy leaves were similar to those obtained with *Helminthosporium victoriae* except that the differences were not so great nor so constant.

TABLE 2.—*Effect of extracts from Helminthosporium victoriae culture, Puccinia coronata- and H. victoriae-infected and healthy oat leaves on cuttings of 16 oat varieties*

Variety and reaction to *H. victoriae*	Degree of damage[a] to cuttings when placed in extracts from									
	Water check	*H. victoriae* culture	Victoria leaves		Bond leaves		Gopher leaves		Marion leaves	
			Infected by *H. victoriae*	Healthy	Infected by *P. coronata*	Healthy	Infected by *P. coronata*	Healthy	Infected by *P. coronata*	Healthy
Susceptible										
Victoria	0	4	5	2	3	1	2	1	2	1
Vicland	0	4	5	3	3	2	2	2	3	2
Osage	0	5	5	2	5	1	2	2	3	2
Neosho	0	3	5	2	4	2	1	0	1	1
Overland	0	5	5	2	4	1	2	2	2	2
Stanton	0	3	5	2	5	2	3	1	3	2
Traveler	0	4	5	2	3	1	2	1	3	2
Fultex	0	5	5	3	3	2	2	2	3	2
Victorgrain	0	5	4	2	2	1	2	2	3	2
Beacon	0	3	4	2	3	2	2	2	3	2
Quincy Gray	0	4	5	2	4	3	2	2	3	1
Quincy Red	0	4	4	2	2	1	2	1	2	1
Garry	0	4	5	2	3	2	3	3	3	2
Av.	0	4.1	4.8	2.2	3.4	1.6	2.1	1.6	2.6	1.7
Resistant										
Marion	0	1	2	3	2	3	3	2	2	3
Bond	0	1	1	2	2	1	3	1	2	2
Gopher	0	2	2	3	4	3	3	3	4	3
Av.	0	1.3	1.7	2.7	2.7	2.3	3.0	2.0	2.7	2.7

[a] Numerical ratings: 0, uninjured; and 5, completely wilted and tissue collapse extensive.

Damage to cuttings from varieties possessing the hypersensitive resistance to crown rust of Victoria in extracts from healthy Bond leaves was consistently less or about one-half that in extracts from leaves infected with *P. coronata*. The extracts from leaves of Gopher and Marion infected with crown rust furnished less striking evidence of a toxic substance in the *P. coronata*-infected plants. Apparently any toxic substance which might have been present in these two varieties was in a lower concentration than in the Bond variety. Differential lodging may have been responsible for the different results obtained. Greater metabolic activity of the pathogen would be expected in the erect Bond plants than in the lodged Gopher and Marion plants.

TITER REQUIREMENTS FOR INJURY BY HELMINTHOSPORIUM VICTORIAE EXTRACT

In order to determine the minimum active dosage of extracts from *Helminthosporium victoriae* with cuttings, a basic concentrate was prepared from pure culture and diluted with distilled water to 0, 5, 10, 25, 50, 75, and 100 per cent concentrations (Series I). In another series (Series II) 0, 0.5, 1, 2, . . . 14, and 15 per cent dilutions were prepared to determine the lower dosage range. Bond and Victoria cuttings were placed in each concentration. Leaves of Victoria plants in dilutions more concentrated than 25 per cent began to wilt in about 20 hours. Simultaneously, localized areas of leaf tissues collapsed. The rapidity of wilting was to a great extent correlated with concentration as was also the rapidity of streaking and yellowing or bronzing. The symptoms appeared much more rapidly at higher concentrations. Victoria cuttings succumbed in all dilutions including the 0.5 per cent concentration.

No important color change was observed in Bond cuttings. Wilting was observed in the cuttings, especially in the more concentrated solutions, but not to the extent observed in the Victoria cuttings. Wilting was correlated with amount of material absorbed, but no direct measurements were made to determine the exact amount. It also was noted that less solution was absorbed by both varieties as the concentration of the extract was increased.

The symptoms observed in the lowest dilutions were those generally observed under natural conditions, especially with older plants, indicating that the toxin concentration in cells of older plants affected would be at the dilution of about 0.5 per cent of the basic concentration of *Helminthosporium victoriae* culture. Concentrations above this point undoubtedly are responsible for the darkening of the tissue before bronzing or yellowing can become effective.

EFFECT OF HELMINTHOSPORIUM VICTORIAE EXTRACT ON TRANSPIRATION

In previous experiments it had been observed that not all dilutions of extracts from *Helminthosporium victoriae* were absorbed equally by Vic-

toria and Bond cuttings. In order to determine how much effect the extract had on absorption or transpiration, uniform lots of culms (4 to 6 weighing 10 gm.) were inserted through holes in cork stoppers into flasks containing 0, 5, 50, and 100 per cent dilutions of the basic concentration. They were then sealed with cotton and paraffin to prevent loss of water by evaporation and placed under three 200-watt Mazda bulbs for observation. At frequent intervals the flasks were weighed to determine the amount transpired.

A summary of results under these favorable conditions for transpiration are presented graphically for the 0, 5, and 100 per cent dilutions in figure 3. The substance or substances contained in the filtrate produced

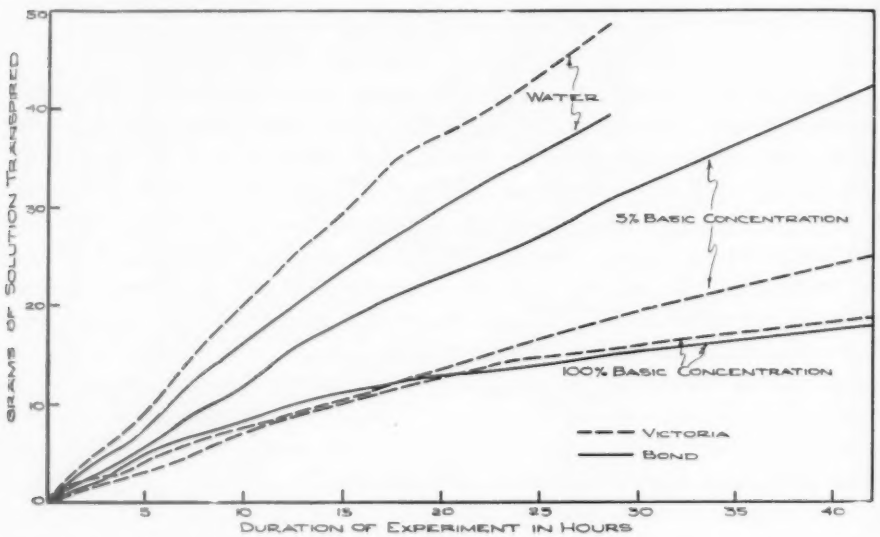

FIG. 3. Effect of extracts from *Helminthosporium victoriae* on transpiration of Bond and Victoria oat cuttings.

two distinct effects upon the plant cuttings: (1) a reduction in transpiration of both varieties and (2) rapid wilting with chlorosis and ultimate death of the Victoria variety. Depression of transpiration rate in the oat cuttings was generally in proportion to the amount of basic extract contained in the solution. The reduced transpiration was also associated with visual wilting. Wilting was more apparent with Victoria cuttings than with Bond because of the extensive injury to the tissues.

The injurious effect was accompanied by loss of rigidity of all tissues of the susceptible variety so that they appeared as though frozen. The appearance of this symptom and rapidity of death were correlated with the concentration of the basic extract. In the concentrated extract, wilting and chlorosis were observed in less than 5 hours, while in the 50 per cent dilution similar symptoms were observed in 6 hours.

To study the effect of extract on transpiration at low and high humidi-

ties, an experiment similar to the previous one was set up. No special attempt was made to reduce the humidity below that prevailing in the laboratory (about 55 per cent), while a humidity of about 90 per cent was maintained in a large germination chamber.

Results are recorded in table 3, and representative flasks are in figure 4 of the experiment. At a humidity of 55, the results were essentially the same as those obtained in the previous experiment, transpiration in both varieties being depressed more or less in proportion to the concentration of the extract present in the absorbed solution. Cuttings kept in the high humidity chamber transpired only about one-seventh as much as those kept at low humidity. The transpiration trends, however, were similar.

TABLE 3.—*Effect of different concentrations of Helminthosporium victoriae culture extracts on transpiration of Victoria and Bond oat cuttings under favorable and unfavorable conditions for transpiration*

Time of exposure	Transpiration from plants in							
	Water		1 per cent basic conc.		5 per cent basic conc.		100 per cent basic conc.	
	Clinton	Tama	Clinton	Tama	Clinton	Tama	Clinton	Tama
(*hr.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)
Low relative humidity with intense light (three 200-watt lamps)								
7	7.7	10.8	8.2	8.2	8.8	4.0	6.5	2.8
19	19.0	25.9	20.7	15.6	19.6	12.4	9.1	6.1
25	22.9	29.7	24.9	19.1	22.0	13.9	9.1	6.2
32	26.4	33.4	28.4	21.4	23.9	16.2	9.4	6.6
44	36.4	39.9	36.1	30.0	28.7	20.4	10.9	7.9
High relative humidity with moderate light (one 200-watt lamp)								
3	0.90	0.90	.....	.....	0.95	0.50	0.80	0.65
11	2.70	2.25	.....	.....	2.23	1.43	1.87	1.57
18	3.82	3.06	.....	.....	3.15	1.82	2.33	2.27
25	4.76	3.82	.....	.....	3.97	2.18	2.71	2.99
37	6.42	5.52	.....	.....	5.61	3.38	3.50	4.67
44	7.18	6.45	.....	.....	6.44	4.33	3.96	5.36

In either case treatments with higher concentrations of the extract reduced transpiration. Wilting, chlorosis, and death of cuttings of the susceptible Victoria variety developed more slowly at high humidity than under conditions less favorable for transpiration.

Because in the preceding experiments reduction in transpiration and subsequent wilting might have been the result of physical plugging of the vascular system at the base of the cuttings, seedlings of Clinton and Tama were cut and placed in extracts containing either 0, 5, or 100 per cent of the basic *Helminthosporium victoriae* concentration for either 5 or 10 hours. At the end of these periods, an additional one-fourth inch was cut off one-half of the culms in each group to remove any vascular obstructions that might have developed during the initial exposure. Then all plants were transferred to flasks of fresh tap water, sealed, and weighed. The

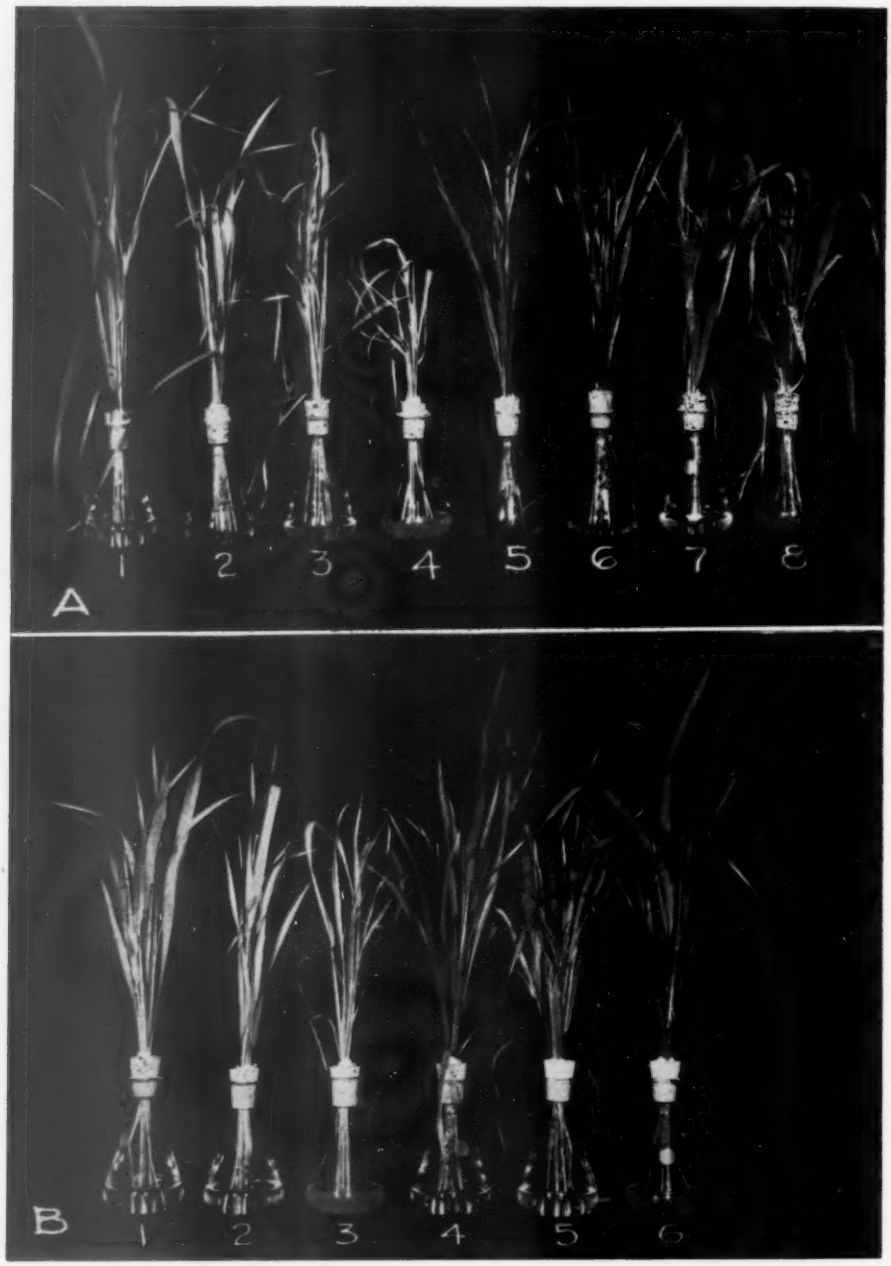

FIG. 4. Cuttings of Bond and Victoria in *Helminthosporium victoriae* extracts under low and high humidity conditions. A, low humidity: 1. Victoria in water. 2. Victoria in 1 per cent concentration. 3. Victoria in 5 per cent. 4. Victoria in 100 per cent. 5. Bond in water. 6. Bond in 1 per cent. 7. Bond in 5 per cent. 8. Bond in 100 per cent. B, high humidity: 1. Victoria in water. 2. Victoria in 5 per cent concentration. 3. Victoria in 100 per cent. 4. Bond in water. 5. Bond in 5 per cent. 6. Bond in 100 per cent.

losses in weight of the cuttings through transpiration were compared for recovery determinations.

Results from this study are in table 4. The *Helminthosporium victoriae*-susceptible Tama cuttings were damaged at both concentrations and they failed to recover when transferred to tap water. The leaves were killed with solutions containing the basic concentration and only the stems remained undesiccated. Injury to the cuttings resembled the immediate effects of frost injury. The 5 per cent dilution had less severe effects on the cuttings, primarily yellowing and bronzing and collapse of tissue in localized areas. Transferring cut and uncut culms to water did not affect

TABLE 4.—*Summary of transpiration data comparing cut and uncut stems of Clinton and Tama oats following initial treatment with extract from Helminthosporium victoriae*

Initial treatment		Basal treatment of stem after initial treatment	Transpiration from cuttings			
			Continuously in toxin extract		Removed to water	
Time of exposure	Material		Clinton	Tama	Clinton	Tama
(*hr.*)			(*gm.*)	(*gm.*)	(*gm.*)	(*gm.*)
5	water	cut	10.38	12.83	11.76	14.71
		uncut	9.67	8.34	10.63	10.89
	5 per cent extract	cut	8.69	5.43	8.62	5.44
		uncut	9.06	4.88	7.38	4.00
	100 per cent extract	cut	4.74	4.84	2.41	4.41
		uncut	4.19	4.26	2.40	3.66
10	water	cut	18.73	24.21	16.46	26.02
		uncut	24.41	29.74	24.48	30.93
	5 per cent extract	cut	16.06	10.47	15.57	12.11
		uncut	18.01	8.93	13.31	8.43
	100 per cent extract	cut	7.13	7.58	2.84	5.43
		uncut	6.43	5.27	2.72	3.56

the transpiration rate, as initially established by the extract treatments.

The resistant Clinton cuttings, on the other hand, were injured much less severely by the extract. After treatment with the basic extract some localized areas of collapsed leaf tissue were observed. These plants did not recover from injury sustained in the initial extract treatment. The lower leaves remained wilted and the rate of transpiration remained at the reduced level even after transfer to water. This depression in Clinton cuttings indicated the injurious effects were from absorbed substances contained in *Helminthosporium victoriae* extract rather than from any possible basal plugging.

EFFECT OF EXTRACTS ON SEEDLINGS GROWING IN SOIL EXTRACT AGAR

Six seeds of Clinton and Victland oats were transferred to hardened agar in each of several beakers. The soil extract agar contained dilutions

of 0, 5, 25, 50, and 75 per cent of the basic extract from *Helminthosporium victoriae* to study effects of the extract on germinating and developing seedlings. The stand was later thinned to five plants per beaker.

Fresh root and top growth weights of the developing seedlings are reported in table 5. Representative plants from comparative treatments are shown in figure 5. Toxic material contained in the extract depresses root development as well as top growth, the depression being striking with the Vicland seedlings. The Vicland seedlings were practically destroyed in the 75 per cent dilution. The 5 per cent concentration was little better, where length of roots averaged 6 mm. and plant height 131 mm. as compared to the water check where root length was 165 mm. and top growth

TABLE 5.—*Effect of different concentrations of Helminthosporium victoriae culture extracts on developing seedlings of Clinton and Vicland in sterile soil extract agar*

Conc. of extract	Variety	Root length	Plant height	Fresh weights of plants		
				Roots	Tops	Total
(%)		(*mm.*)	(*mm.*)	(*gm.*)	(*gm.*)	(*gm.*)
None (control)	Clinton	155	230	.18	.75	.93
	Vicland	165	220	.20	.75	.93
5[a]	Clinton	85	275	.21	.88	1.09
	Vicland	7	132	.07	.36	.43
5[a]	Clinton	72	250	.25	.85	1.10
	Vicland	5	130	.07	.38	.45
25	Clinton	63	225	.33	.94	1.27
	Vicland	3	40	.03	.20	.23
50	Clinton	60	230	.30	.56	.86
	Vicland	2	30	.02	.14	.16
75	Clinton	45	210	.10	.71	.81
	Vicland	0	25	.01	.06	.07

[a] These two treatments differ in the preparation of the culture medium, the upper having been prepared with 50 per cent, while the lower was prepared with 95 per cent of soil extract agar.

was 220 mm. Fresh green weights were closely correlated with physical measurements. The effect of the extract on Clinton was mild as compared to the effect on the *Helminthosporium victoriae*-susceptible Vicland. Only with the 50 and 75 per cent dilutions was there any appreciable reduction in growth of seedlings. The roots of Clinton, like those of Vicland in the water control, were normal for color, while Vicland roots in presence of the toxic extract were distinctly light brown.

In another experiment Clinton and Vicland were again grown in soil extract agar with extracts from *Puccinia coronata*-infected plants, *Helminthosporium victoriae*-infected plants, *H. victoriae* culture (5 per cent dilution), healthy plants, and water. Data on the various extracts (Table 6) confirm the observations in preceding trials with other methods. Tox-

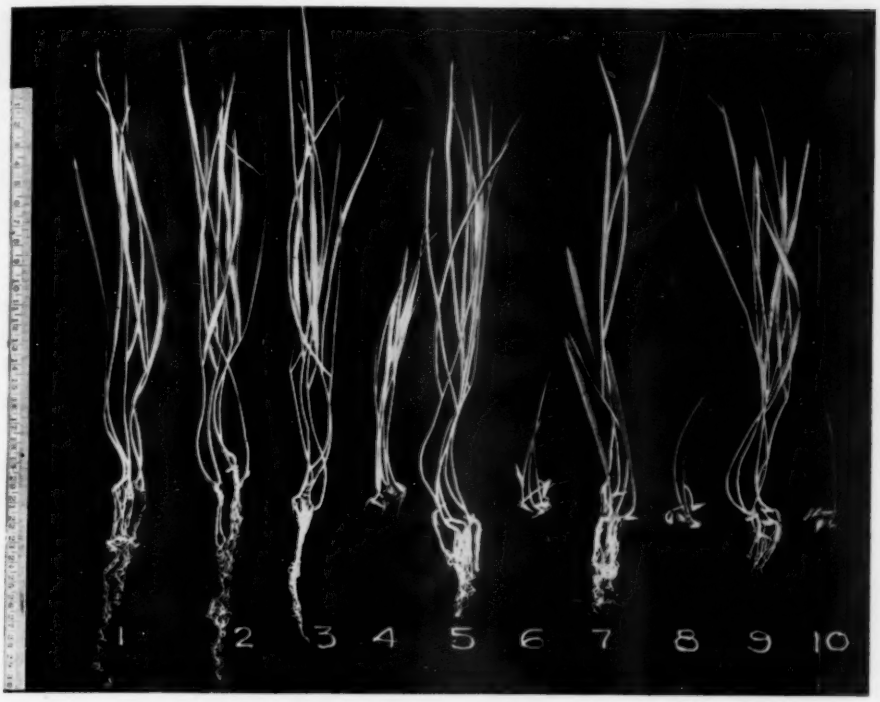

FIG. 5. Seedlings of Clinton and Vicland grown in sterile soil extract agar to which were added extracts from *Helminthosporium victoriae* culture. 1. Clinton in water. 2. Vicland in water. 3. Clinton in 5 per cent extract. 4. Vicland in 5 per cent. 5. Clinton in 25 per cent. 6. Vicland in 25 per cent. 7. Clinton in 50 per cent. 8. Vicland in 50 per cent. 9. Clinton in 75 per cent. 10. Vicland in 75 per cent.

icity or depression as evidenced by reduced root and top growth was obtained with Victoria seedlings from the *H. victoriae* culture extract as well as from extracts from plants infected with *H. victoriae* or *P. coronata*. The plant extracts appeared to depress growth in seedlings of both varie-

TABLE 6.—*Effect of extracts from Helminthosporium victoriae culture, H. victoriae-infected plants, Puccinia coronata-infected plants, and healthy plants on developing seedlings of Clinton and Vicland in sterile soil extract agar*

Source of extract	Variety	Root length	Plant height	Fresh weights of plants		
				Roots	Tops	Total
		(*mm.*)	(*mm.*)	(*gm.*)	(*gm.*)	(*gm.*)
None	Clinton	124	233	.35	.86	1.21
	Vicland	107	207	.20	.66	.95
Healthy plants	Clinton	23	167	.15	.51	.66
	Vicland	18	172	.14	.47	.61
P. coronata-infected plants	Clinton	20	167	.14	.47	.61
	Vicland	14	157	.10	.43	.53
H. victoriae culture	Clinton	93	250	.23	.85	1.08
	Vicland	28	172	.11	.38	.50
H. victoriae-infected plants	Clinton	26	182	.14	.61	.75
	Vicland	14	182	.10	.47	.57

ties, but with Vicland the healthy plant extract was consistently less damaging to developing seedlings than were extracts from diseased plants. It could, therefore, be concluded that in addition to the depressing effects of plant extracts a toxic principle operated in Victoria seedlings, as has been found by previous methods of comparison. Root development, probably the best index for measuring the prevalence of the toxin, was consistently less with the plant extract from diseased plants.

EFFECT OF EXTRACTS ON SEEDLINGS GROWING IN SAND

Cultures in sterile sand with extracts of *Helminthosporium victoriae* and *H. victoriae*-infected plants, *Puccinia coronata*-infected plants, healthy plants, and water were made with Clinton and Vicland. Care was necessary in applying water to prevent waterlogging. In table 7 results ob-

TABLE 7.—*Effect of extracts from Helminthosporium victoriae culture, H. victoriae-infected plants, Puccinia coronata-infected plants and healthy plants on developing seedlings of Clinton and Vicland in unsteamed sand*

Source of extract	Variety	Root length	Plant height	Fresh weights of plants		
				Roots	Tops	Total
		(*mm.*)	(*mm.*)	(*gm.*)	(*gm.*)	(*gm.*)
None (control)	Clinton	100	225	.54	.72	1.26
	Vicland	110	210	.50	.75	1.25
Healthy plants	Clinton	75	210	.37	.86	1.23
	Vicland	70	220	.32	.84	1.16
P. coronata-infected plants	Clinton	75	205	.43	.82	1.25
	Vicland	75	175	.23	.58	0.81
H. victoriae culture (1 per cent conc.)	Clinton	85	230	.45	.69	1.14
	Vicland	70	220	.36	.74	1.10
H. victoriae culture (5 per cent conc.)	Clinton	100	240	.39	.80	1.19
	Vicland	75	200	.32	.68	1.00
H. victoriae-infected plants	Clinton	70	210	.34	.81	1.15
	Vicland	70	210	.32	.81	1.13

tained with these different extracts are given. Depression in developing Vicland seedlings as evidenced by reduced root and top growth was obtained with extracts from *P. coronata* and *H. victoriae*. The data confirm those obtained with the soil extract agar method except that differences were less striking. It is entirely possible that in the presence of soil organisms the toxicity of the extracts may be reduced.

In another experiment eight seeds each of Clinton and Vicland were grown in steamed and nonsteamed sand to which were added *Helminthosporium victoriae*, extracts from the mycelial growth, and water. Five grams of sterile Webster loam soil were mixed with the contents of each beaker to supply additional nutrient to the growing seedlings. The data in table 8 verify observations made previously.

When viable *Helminthosporium victoriae* was placed with seeds of Vicland and Clinton, no Vicland plants survived. They were killed either

before or after emergence. Some seedlings of Clinton failed to emerge. When examined they were alive but folded up without the protection of the coleoptile. This reaction had been observed in greenhouse tests with the viable organism.

EFFECT OF HYPODERMIC INJECTION OF EXTRACTS ON GROWING PLANTS

Portions of the extracts were injected by hypodermic needle, into numerous stems of growing plants of both Bond and Victoria. Subsequent readings on the Victoria plants gave positive readings with the basic concentration from *Helminthosporium victoriae*. The symptoms observed were characteristic streakings of the leaves above the point of injection as is normally observed under natural conditions. The extract from *Puccinia coronata*- and *H. victoriae*-infected plants gave only weak streaking

TABLE 8.—*Effect of Helminthosporium victoriae and 5 per cent extracts from it on developing seedlings of Clinton and Vicland oats in steamed and unsteamed sand*

Treatment	Variety	Root length	Plant height	Fresh weights		
				Roots	Tops	Total
		(*mm.*)	(*mm.*)	(*gm.*)	(*gm.*)	(*gm.*)
None (control)	Clinton	100	225	.54	.72	1.26
	Vicland	110	210	.50	.75	1.25
Extract in steamed sand	Clinton	57	190	.27	.74	1.01
	Vicland	30	148	.17	.56	.72
Extract in unsteamed sand	Clinton	85	228	.29	.79	1.08
	Vicland	110	210	.34	.75	1.09
Viable *H. victoriae* on seed at sowing	Clinton	58	195	.41	.74	1.15
	Vicland[a]	.....	.....	.....	.....	.....

[a] No plants survived; death occurred before or after emergence. The root development varied in accordance with the development of the plumule.

symptoms on Victoria, while other treatments and other varieties resulted only in localized necrotic lesions where the needle penetrated.

HYPODERMIC INJECTION AND NEEDLE DEPOSITION OF VIABLE HELMINTHOSPORIUM VICTORIAE MYCELIUM

Viable mycelial suspensions were injected into oat culms by hypodermic needle. When stems thus inoculated were placed in the moist chamber for incubation, infection occurred on both susceptible and resistant varieties. Very small quantities of inoculum were necessary; in fact, puncturing the stem with the needle of the filled syringe seemed to be sufficient. Although infection was obtained in both varieties, only Victoria stems were killed by the pathogen. Only the tissues in the immediate vicinity of the point of inoculation or those above the point of infection were killed. To kill the entire plant, infection must occur in a more vital part than above the soil line on one of the tillers.

Uniform amounts of mycelium were inserted into oat stems with an or-

dinary dissecting needle to study movement of the toxic substance produced by the fungus. Infection was obtained in both resistant and susceptible varieties, but only tissues of the susceptible variety were destroyed by the toxin produced by the pathogen. In no case was there any apparent downward movement of the toxic substance nor was there any apparent movement into other tillers of the same plant except where the point of inoculation was very close to the ground line so that the fungus could penetrate the crown region of the plant.

Resistant varieties (Bond, Clinton, Ukraine, Iogold, Hajira × Joanette, and Markton) tested by this extreme method were not affected by the toxic substance produced by *Helminthosporium victoriae* even though the pathogen could be observed growing at the point of injection. In several instances individual stems died, but this apparently resulted from mechanical injury caused by the inoculating needle.

Stems of Victoria and Bond were inoculated with mycelium of several other species of *Helminthosporium*, but the effects were similar to those obtained with *H. victoriae* on varieties resistant to it.

DISCUSSION

Data have been presented in this paper to show that *Helminthosporium victoriae* produces a toxic substance (or substances) which reduces transpiration in both resistant and susceptible varieties of oats, and, in susceptible varieties, strikingly reduces growth and also destroys cells. The exact nature of this active material or toxin is not known, but its effects are so distinctive that there can be little doubt that it contributes to chlorosis and striping symptoms observed in plants infected with *H. victoriae*. The material is so potent that it is effective at very low dosages.

The presence of a toxin was demonstrated in diseased plant tissues at concentrations sufficient to affect the oat plant. There were some conflicting data on the plant extracts, since extracts from normal plants also yield injurious materials. However, infected plants yielded extracts with toxic effects consistently greater than those of extracts from normal plants.

The present concept is that the active ingredient contributes materially to the parasitism of *Helminthosporium victoriae*. The fungus is a facultative saprophyte, so the presence of injured tissue undoubtedly stimulates its more extensive development.

Some evidence was obtained of the presence of a comparable toxic substance in plants infected with *Puccinia coronata*. It cannot be maintained that the two fungi produce identical toxins, since neither has been purified. The toxins behave in a comparable fashion, however, as shown by a number of different trials with extracts from these two organisms.

The evidence indicates that the toxic substance from *Puccinia coronata* is released at about the time the fungus begins to sporulate. This coincides with the period when the host undergoes a hypersensitive reaction and sufficient cells collapse to restrict further development of the rust

fungus. The release of metabolic by-products at this time is in keeping with observations of Welsh (5) with *Actinomyces*, a fungus which releases antibiotic substances at the time of sporulation.

The evidence strongly suggests that *Helminthosporium victoriae* and *Puccinia coronata* produce comparable toxins and that further parasitic development depends upon differences in the innate abilities of the two fungi. The dead and injured cells probably serve as a readily available source of food for the facultative saprophyte and as a physiological barrier to an obligate parasite such as *P. coronata*. If this reasoning is correct then the hypersensitive reaction to *P. coronata* can be attributed to the toxins produced.

Very strong supporting genetic evidence for the presence of a gene with pleiotropic effects has been obtained by the author in inheritance studies of crosses involving resistance and susceptibility to these two pathogens. No plants were observed in thousands tested where the hypersensitive type of reaction to *Puccinia coronata* was separated from susceptibility to *Helminthosporium victoriae*. Resistance to crown rust of the hypersensitive type possessed by Victoria always was associated with susceptibility to *H. victoriae* and is apparently dependent upon the gene for susceptibility to this pathogen for its expression. In *H. victoriae*-susceptible germ plasm, resistance to crown rust of this type can be expressed, while in its absence plants are susceptible unless there are other factors present for resistance which inhibit the normal development of the crown rust pathogen.

CONCLUSIONS

1. Evidence was obtained that *Helminthosporium victoriae* in culture produces a toxic substance that, at very low dilutions, affects oat plants.

2. In Victoria plants the toxic extract of *H. victoriae* produces rapid chlorosis, wilting, and necrosis, the chlorosis and necrosis being specific to the susceptible genotypes.

3. The toxic extract reduces transpiration in both resistant and susceptible varieties. Injured plants do not recover from this traumatic effect.

4. Toxins were detected from *H. victoriae*-infected plants in concentration sufficient to bring about effects similar to those obtained with the pure culture.

5. Extracts from plants infected with *Puccinia coronata* produced a similar effect on genotypes with a hypersensitive reaction. The extracts from healthy plants also were toxic to these plants but the injury was not so severe. It is believed that *P. coronata* produces a toxin in sufficient concentration by the time of sporulation to induce the localized necrosis described as a hypersensitive reaction.

6. It is suggested that susceptibility to *H. victoriae* and resistance to *P. coronata* depend upon comparable types of reactions induced by

toxins. The destruction of host tissue by toxins would facilitate growth of a facultative saprophyte such as *H. victoriae* while providing a physiological barrier to an obligate parasite such as *P. coronata*. The hypothesis of a gene with pleiotropic effects is in agreement with available genetical data.

IOWA STATE COLLEGE,
AMES, IOWA

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4. MURPHY, H. C., and FRANCES MEEHAN. Reaction of oat varieties to a new species of *Helminthosporium*. (Abstr.) Phytopath. **36**: 407. 1946.
5. WELSH, M. Bacteriolytic properties of *Actinomyces*. Rept. Proc. Third Intern. Congr. Microbiol. **1939**: 260–261. 1940.

ANNOUNCEMENT

PROPOSED NEW EDITIONS OF OUT-OF-PRINT MYCOLOGICAL WORKS

With the current interest in the taxonomy and nomenclature of the fungi a pressing need has arisen for certain basic works on the subject which have long been out of print and of which even the few available copies are showing signs of wear and tear. Four outstanding items have been selected for reproduction by the photo-offset process provided sufficient advance subscriptions are indicated. The works in question together with the probable price per volume or set follow.

FRIES, E. *Systema Mycologicum.* The three volumes together with the *Elenchus Fungorum* and the index to the complete work	$ 50.00
PERSOON, C. H. *Methodica Fungorum*	20.00
LINDAU, G., and SYDOW, P. *Thesaurus Mycologicae et Lichenologicae.* 5 volumes	90.00
ZAHLBRUCKNER, A. *Catalogus Lichenum Universalis.* 10 volumes with index	175.00

The first and second of these are the classical works on which the nomenclature of the fungi is based under the International Rules. They have not been available even as second-hand copies for many years. The third lists the literature of mycology and plant pathology up to 1910 and is invaluable to all working on the historical aspects of any problem in these fields. The final item, which is to the lichenologist what Saccardo's *Sylloge Fungorum* is to the Mycologist, is out of print and unobtainable.

In order that an idea of the number of prospective sales may be obtained, it is requested that tentative subscriptions, either personal or institutional, be sent to the undersigned.—JOHN A. STEVENSON, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

REPORT OF THE 40TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its fortieth annual meeting at the William Penn Hotel in Pittsburgh, Pennsylvania, on December 6, 7, and 8, 1948. The Northeastern Division met with the parent society. The North Central Division held an informal meeting. On the afternoon of December 8, a joint session was held with the Potato Association of America. Three hundred and eighty-seven members registered from forty states and six foreign countries. One hundred and twenty-three papers were presented in seventeen sessions and symposia. Conferences and symposia were held under the titles of "The Teaching of Plant Pathology; Extension Workers' Conference; Nature and Methods of Inspection of Nursery Stock for Importation and Interstate Shipment; Stone Fruit Virus Disease Problems; and Fungicide Colloquium."

The annual dinner held at the William Penn Hotel was attended by approximately three hundred. The entertainment at the dinner by the Westinghouse male quartet was received enthusiastically.

Council for 1949:

W. D. VALLEAU, President (1 yr.), Kentucky Agricultural Experiment Station, Lexington 29, Kentucky
C. M. TUCKER, Vice-President (1 yr.), University of Missouri, Columbia, Missouri
CURTIS MAY, Secretary (3-yr. term expires 1950), Plant Industry Station, Beltsville, Maryland
M. C. RICHARDS, Treasurer and Business Manager of PHYTOPATHOLOGY (3-yr. term expires 1949), University of New Hampshire, Durham, New Hampshire
HELEN HART, Editor-in-Chief, PHYTOPATHOLOGY (3-yr. term expires 1951), University Farm, St. Paul 1, Minnesota
W. F. BUCHHOLTZ, Iowa State College, Ames, Iowa
S. J. P. CHILTON, Louisiana State University, University, Louisiana
L. C. COCHRAN, Citrus Experiment Station, Riverside, California
R. S. KIRBY, Pennsylvania State College, State College, Pennsylvania
S. G. LEHMAN, North Carolina State College, Raleigh, North Carolina
S. E. A. MCCALLAN, Boyce Thompson Institute for Plant Research, 1086 North Broadway, Yonkers, New York
PAUL R. MILLER, Plant Industry Station, Beltsville, Maryland
W. H. TISDALE, Du Pont Experiment Station, Wilmington 98, Delaware

Representatives:

A.A.A.S. Council. R. S. KIRBY, H. A. RODENHISER
Division of Biology and Agriculture, National Research Council. J. C. WALKER
American Institute of Biological Sciences. J. C. WALKER
American Type Culture Collection. H. H. MCKINNEY
Board of Editors, American Journal of Botany. C. C. CHUPP
Scientific Liaison and Advisory Board, Quartermaster Food and Container Institute for the Armed Forces. H. H. THORNBERRY

Standing Committees:

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Extension: O. C. BOYD, Chm.; H. R. GARRISS; L. O. WEAVER; R. J. HASKELL; S. B. FENNE; E. G. SHARVELLE; J. E. LIVINGSTON; J. O. ANDES; C. E. SCOTT; C. F. BISHOP
Investments: M. C. RICHARDS, Chm.; E. L. LECLERG; MARVIN E. FOWLER; R. M. CALDWELL
Phytopathological Classics: L. J. TYLER, Business Manager; L. C. KNORR, Editor
Placement: R. S. KIRBY, Chm.; H. A. RODENHISER; M. W. GARDNER; R. J. HASKELL; L. M. MASSEY; S. J. P. CHILTON
Public Relations: GEORGE MCNEW, Chm.; PAUL R. MILLER; K. S. CHESTER; L. S. HITCHNER; J. D. MOORE; GEORGE ZENTMEYER; HARRY O'BRIEN; C. E. COX; R. R. KINCAID; O. D. BURKE; F. J. GREANEY
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Plant Disease Prevention: J. G. LEACH, Chm.; PAUL R. MILLER; S. B. FRACKER; E. C. STAKMAN; L. C. COCHRAN; H. H. THORNBERRY; C. R. ORTON

Subcommittee on Regulatory Work and Foreign Plant Diseases: C. R. ORTON, Chm.; R. P. WHITE; W. H. WHEELER; E. G. REX
Subcommittee on Seed and Plant Material Certification: L. C. COCHRAN, Chm.; REINER BONDE; CHARLES CHUPP; C. H. ARNDT; H. C. MURPHY
Subcommittee on Utilization of Plant Pathologists and Field Facilities in National Emergencies: H. H. THORNBERRY, Chm.; LESLIE WALDEE; H. T. COOK; H. P. BARSS; A. L. ANDERSEN

Special Committees:

Fungicides: J. D. WILSON, Chm.; C. H. ARNDT; F. L. HOWARD; J. W. HEUBERGER; DWIGHT POWELL
Subcommittee on Seed Treatments: C. H. ARNDT, Chm.; J. E. MACHACEK; P. E. HOPPE; COYT WILSON; E. D. HANSING; K. F. BAKER; A. G. NEWHALL
Subcommittee on Dusts and Sprays: J. D. WILSON, Chm.; J. M. HAMILTON; A. W. DIMOCK; J. W. HEUBERGER; W. F. BUCHHOLTZ; H. F. WINTER; M. B. LINN
Subcommittee on Special Problems: F. L. HOWARD, Chm.; L. S. HITCHNER; S. E. A. MCCALLAN
Subcommittee on Annual Summary of Results on Newer Fungicides: J. W. HEUBERGER, Chm.; PAUL R. MILLER; E. E. CLAYTON; M. C. GOLDSWORTHY; R. W. LEUKEL; R. J. HASKELL; W. D. MCCLELLAN
Subcommittee on Fungicide Nomenclature: DWIGHT POWELL, Chm.; J. D. MOORE; W. H. TISDALE; J. C. DUNEGAN; E. A. WALKER; S. L. HOPPERSTEAD
Fungus Nomenclature: J. A. STEVENSON, Chm.; W. W. RAY; W. C. SNYDER; D. E. ELLIS; C. M. CHRISTENSEN
Nomenclature and Classification of Plant Viruses: L. M. BLACK, Chm.; W. C. PRICE; FREEMAN WEISS; C. W. BENNETT; H. H. MCKINNEY; JAMES JOHNSON; FRANK MCWHORTER
Subcommittee on Virus Type Culture Collection: H. H. MCKINNEY, Chm.; JAMES JOHNSON; T. J. GRANT
Membership: C. M. TOMPKINS, Chm.; W. J. JEFFERS; T. W. BRETZ; B. H. DAVIS; C. N. CLAYTON; P. M. SIMMONDS; THOR KOMMEDAHL; GLENN S. POUND; L. K. WRIGHT; M. C. RICHARDS, ex officio
Sustaining Associates: E. G. REX, Chm.; P. P. PIRONE; P. D. PETERSON
Nomenclature of Plant Pathogenic Bacteria: O. N. ALLEN, Chm.; J. G. LEACH; W. H. BURKHOLDER; CHARLOTTE ELLIOTT; E. M. HILDEBRAND; P. A. ARK
Teaching of Plant Pathology: G. F. WEBER, Chm.; J. G. LEACH; G. C. KENT; THOMAS SPROSTON, JR.; M. F. KERNKAMP

Temporary Committee for 1949:

Publication of Special Material: DONALD CATION, Chm.; H. P. BARSS; GEORGE KENT; W. H. TISDALE

Report of the Secretary. On December 3, 1948, the total membership was 1,497, consisting of 143 life members, 1,211 regular members, and 143 applicants to be accepted at the Pittsburgh 1948 annual meeting. Nine members died during the year: Cyril O. Bratley (life), Anthony Berg, Loran Blood, B. F. Lutman, William M. Scott, George H. Smith, Albert F. Woods (charter, life), Sanford M. Zeller, and Howard S. Fawcett.

The Society by mail vote of the members approved the proposal to affiliate with the American Institute of Biological Sciences.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1948.

Receipts:

Balance from 1947			$ 4,936.46
Annual dues:			
1947	$ 55.50		
1948	5,069.51		
1949–53	145.75	$5,270.76	
Sales		73.42	
Sustaining Associates		1,600.00	
Transferred from PHYTOPATHOLOGY		1.00	
			6,945.18
Total Receipts			$11,881.64

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY			
1947	$ 45.76		
1948	4,224.57		
1949–53	121.30		
		$4,391.63	
Transferred to PHYTOPATHOLOGY for sales		73.42	
		4,465.05	
Office of Secretary			
Sec. work	90.30		
Stamps	74.15		
Printing	174.01		
Other	17.59	356.05	
Office of Treasurer			
Sec. work	321.90		
Stamps	53.47		
Other	43.29	418.66	
Office of President			
Printing		20.68	
Biological Abstracts		100.00	
Placement Committee		36.55	
Sustaining Associate Committee		22.00	
Annual Meeting			
Rooms	398.05		
Travel	159.81	557.86	
Withholding Tax		24.00	
Total Expenditures			$ 6,000.85
Balance on hand October 30, 1948			5,880.79
			$11,881.64

Report of the Business Manager. The costs of carrying out the functions of the Business Office increased this year over 1947 by $4,552.98. With 1418 members and 907 subscribers there was a total of 2356 copies of PHYTOPATHOLOGY distributed each month during 1948. This is 196 more than in 1947.

Statement of Accounts for the Year Ending September 30, 1948.

Receipts:

Balance on hand from 1947			$ 7,259.93
Subscriptions:			
1947	$ 156.55		
1948	5,646.50		
1949–50	800.30	$6,603.35	
Member subscriptions and sales transferred from APS			
1947	45.76		
1948	4,224.57		
1949–53	121.30		
Sales	73.42	4,465.05	
Sales back numbers of PHYTOPATHOLOGY		1,927.91	
Sales of Membership list		6.00	
Sales of 30-year index		99.15	
Reprints		1,552.28	
Excess illustrations		269.60	
Rockefeller Institute Contribution		600.00	
Interest on sinking funds		86.50	
Donations and Legacies fund		52.00	
Unused credit		55.95	
Excess abstracts		2.80	
Refunds		17.82	
Transfer to APS		1.00	
Advertising		1,917.19	
Total Receipts			17,656.60
Bank balance 1947 and receipts for 1948			24,916.53
Northwestern Federal Savings and Loan			5,340.54
Grand Total			$30,257.07

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY			
Vol. 37, no. 9	$ 891.33		
10	901.65		
11	891.66		
12	1,144.81		
Vol. 38, no. 1	1,005.52		
2	843.49		
3	1,201.56		
4	987.10		
5	1,073.30		
6	1,051.90		
7	1,146.91		
8	1,028.18		
Postage	881.69		
Engravings	876.09	$13,925.19	
Office of Business Manager			
Secretary	761.91		
Supplies	53.48		
Stamps	33.01		
Miscellaneous	159.08	1,007.48	
Editor-in-Chief			
Secretary	1,399.15		
Supplies	106.75		
Express	7.75		
Miscellaneous	9.39	1,523.04	
Advertising Manager			
Secretary	72.00		
Postage	11.64		
Commission	135.67		
Miscellaneous	30.28	249.59	
Purchase of back volumes		31.00	
Refunds		116.47	
Express		16.51	
Printing		367.91	
Miscellaneous		40.66	
News Letter		87.70	
Bank charges		1.03	
Reprints		1,229.32	
Transfer APS dues		1.00	
Membership list		616.99	
Total Expenditures			$19,213.89
Balance on hand:			
Checking account		5,702.64	
Northwestern Federal Savings and Loan		5,340.54	11,043.18
Grand Total			$30,257.07

Sinking Fund. The principal amount of the sinking fund remained the same as for the previous year.

First mortgage note, at 3.8 per cent interest, deposited with McLachlen Banking Corporation for collection	$ 500.00
U. S. Savings Bond, Series G, No. M19056026, 2½ per cent interest	1,000.00
Invested with the following:	
Jefferson Federal Savings & Loan Ass'n (accrued dividends, $103.65)	603.65
District Bldg. Loan Association (accrued dividends, $306.66)	1,806.66
National Permanent Bldg. Ass'n (accrued dividends, $517.68)	2,517.68
Northwestern Fed. Savings & Loan Association (certificates)	2,000.00
Perpetual Building Association (accrued dividends, $207.31)	1,207.31
Prudential Building Association (accrued dividends, $44.85)	251.19
Arlington and Fairfax Bldg. & Loan (accrued dividends, $109.80)	1,109.80
	$10,996.29
Less interest due PHYTOPATHOLOGY	1,289.95
	$ 9,706.34

Lyman Memorial Fund. This fund is obtained from voluntary contributions and now totals $3958.94. This amount is invested with the Brookland Building and Loan Association at 2½ per cent interest.

Balance on hand September 30, 1947	$ 3,807.96
Dividends December 31, 1947, and June 30, 1948	96.07
Voluntary contributions	54.91
	$ 3,958.94
Less interest due PHYTOPATHOLOGY	582.21
	$ 3,376.73

Additional Endowment:

War Savings Bonds, Series F (maturity value)	$ 1,125.00
War Savings Stamps (maturity value)	7.00
Total value at maturity	$ 1,132.00

Report of the Auditing Committee for the fiscal year, 1947–1948. We have examined the books of the Treasurer of the American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period from September 30, 1947, to September 30, 1948. We found all receipts and expenditures and all funds of the Society and of PHYTOPATHOLOGY accurately recorded.

Signed: GUS A. MCLAUGHLIN
R. C. COOMBS

Report of the Advertising Manager.

	Number of Advertisements (Paid) Pages	Space Used Pages	Gross Income Dollars	Net Income Dollars
	83	67.5	$2,780.80	$2,457.82
Membership List	11	11	445.00	378.67
Total, 1948	94	78.5	$3,225.80	$2,836.49

Expenses: Stenographic and Miscellaneous—$112.84

Pages of non-paying ads:

Biological Abstracts: ½ page Jan., Feb., Oct., Nov.,	2
Placement Committee: ½ page Jan., July, Sept., Oct., Nov.,	2½
Phytopathological Classics: Apr., May, Sept., Oct., Nov.,	5
Forms for Bequest and Codicil: November	1
Total	10½

Report of the Editor-in-Chief. Volume 38 of PHYTOPATHOLOGY contains 1,040 pages in which 374 authors published 105 long articles, 38 phytopathological notes, 208 abstracts, and 6 book reviews. The report of the annual meeting of the Society was published in the April issue. Seven manuscripts have been withdrawn or rejected since December, 1947. Thirty manuscripts were in press on November 1, 1948, eleven were being revised by authors, and fifteen were being reviewed by the editors. Increased costs of publication have necessitated rigorous scrutiny of articles and numerous requests for condensation of manuscripts.

Report of the Editor, Phytopath News. As of December 1, three numbers of NEWS were published and distributed to members at a cost of $129.57. Another number awaits publication at an estimated cost of $43.26, thus making a total expenditure of approximately $172.83. If six numbers of NEWS are published in the same manner during 1949, a budget of $300.00 will probably suffice.

There are two alternatives for publishing PHYTOPATH NEWS: (1) continuation of the present system whereby NEWS is printed within a two-week period by a local press and is then sent to Lancaster, Pennsylvania for addressing and mailing. The chief advantages of this method are that no definite deadline must be met and that news items reach members with minimum delay. The principal disadvantage is the high cost of

printing and distributing each number. (2) A page in PHYTOPATHOLOGY might be designated for NEWS on a bimonthly basis or more frequently if it seems desirable. The chief advantage of this method would be a considerable saving in cost. Disadvantages include, a minimum period of six to eight weeks or more before news items would reach members and additional lay-out work for the Editor-in-Chief of PHYTOPATHOLOGY.

A number of individuals representing fields of administration, research, extension, and industry have expressed their interest in the new venture, and a few have contributed suggestions for increasing the usefulness of NEWS. The principal difficulty at present is to obtain items for publication. If everyone cooperates in supplying material, this obstacle can be readily overcome.

Report of Representative on Division of Biology and Agriculture, National Research Council. The annual meeting of the Division of Biology and Agriculture, National Research Council, was attended at Washington, D. C., May 6, 1948. A full report was sent to Council members on May 26, 1948. During the year the American Institute of Biological Sciences was launched under the sponsorship of the Division. About 15 societies are now affiliated. A permanent Executive Secretary has been appointed.

Representatives of the Division have participated in the representation of biologists before the government committees concerned with selective service and war manpower.

The Committee on Genetics of Microorganisms is meeting in Washington in November to make future plans.

The Committee on Aerobiology, after necessary quiescence during the war period, is being revivified under the chairmanship of Dr. E. C. Stakman.

Report of Representative on the Governing Board of American Institute of Biological Sciences. The Society voted, by mail ballot, during the year to join the American Institute of Biological Societies as of January 1, 1949. An organization meeting was held at Washington on February 18, 1948; a second meeting was held in Washington on September 13, 1948. Both meetings were attended by Dr. Curtis May as observer. Dr. Milton O. Lee has been appointed as Executive Secretary. Among the committees now in action are one on Selective Service, and one on publications.

The Institute undertook to sponsor the Convention of Biological Societies in Washington in September, 1948, in which eleven societies participated. The Governing Board has instructed the Executive Secretary to work out suitable arrangements with AAAS for 1949 meetings in New York, and to inquire of constituent societies their wishes in regard to meetings in 1950.

Report of Committee on Donations and Legacies. The committee has placed a total of $66.00 with the Treasurer and from this fund $24.00 has been used to provide subscriptions for five foreign plant pathologists. It is suggested that a new chairman be appointed from the eastern area, preferably from near Washington, D. C., because of facilities that are available there for securing needed information.

Report of Committee on Extension. The Committee has sponsored an annual conference of extension plant pathologists the last few years in conjunction with the annual meeting of the American Phytopathological Society. They also have a luncheon meeting of the extension plant pathologists at which time mutual problems are discussed. During the past year the committee has been active on the following projects, which are under supervision of selected personnel of the Extension Committee and act as subcommittees of the Extension Committee: (1) Methods of filing and indexing bulletins, reprints, and other subject matter materials by extension plant pathologists, (2) summary information on airplane dusting and plant disease control in the United States, (3) the three-foot book shelf for extension plant pathologists, and (4) subjects that would be included in the graduate curriculum in training young extension plant pathologists.

Reports of these subcommittees will be presented at our annual conference in Pittsburgh this year and summaries will probably be available for distribution later.

The following is an outline of the program of the extension conference at the Pittsburgh meetings: (1) reports of various subcommittees, (2) present situation in extension plant pathology, (3) opportunities for making contributions to agriculture, (4) progress in extension in varous states, and (5) an open discussion on future plans and other pertinent subjects as requested by members attending.

Report of the Investment Committee. Total investments of the Society for the year ending September 30, 1948, are as follows:

Sinking Fund	$10,996.29
Lyman Memorial Fund	3,958.94
Additional Endowment:	
War Savings Bonds Series F (maturity value)	1,125.00
War Savings Stamps (maturity value)	7.00

The committee requests that Council and members approve the following proposals:

(a) That $11.50 from the general funds of the Society be added to the $7.00 in War Savings Stamps and a Series F War Savings Bond with a maturity value of $25.00 be purchased.

(b) That the War Savings Bonds having a total maturity value of $1,150.00 now carried as Additional Endowment be transferred to the Sinking Fund.

Report of the Editor, Phytopathological Classics. The year 1948 marked issuance of PHYTOPATHOLOGICAL CLASSIC No. 8: Berkeley's "Observations, Botanical and Physiological, on the Potato Murrain," together with selections from his "Vegetable Pathology." The selections were made by the Plant Pathology Committee of the British Mycological Society from articles Berkeley published in the GARDENER'S CHRONICLE AND AGRICULTURAL GAZETTE between the years 1854 and 1857. In this serialization, Berkeley outlined his extensive classification of plant diseases and detailed this with information then extant concerning the various causal fungi. The work, which originally consisted of 173 separate articles, is now brought together in No. 8.

Plans for No. 9 are under way, and the new CLASSIC will be ready for printing when the proceeds from sales of previous issues again make funds available. Under consideration for publication are: (1) Tozetti's Alimurgia, (2) M. Navashin's paper on *Sclerotinia betulae*, (3) selections from the works of Erwin F. Smith, with emphasis on the Smith-Fischer controversy, and (4) a review of recent Russian phytopathological research. Opinion has been solicited on the value of translating and reprinting the Alimurgia, the work in which Count Re sees historically the first real suggestion that plant diseases are caused by specific organisms. Both Dr. P. P. Pirone and Dr. J. C. Walker, who have reviewed the selection made by Professor Gabriele Goidanich, deem the work an interesting and worthwhile addition to PHYTOPATHOLOGICAL CLASSICS.

Suggestions concerning other likely papers for inclusion in the PHYTOPATHOLOGICAL CLASSICS series are always welcome.

Report of the Business Manager, Phyopathological Classics.

Classic No. 1:	On hand, October 1, 1947	out of print	
Classic No. 2:	On hand, October 1, 1947	166	
	Sold during year	19	$ 9.50
	On hand, September 30, 1948	147	
Classic No. 3:	On hand, October 1, 1947	253	
	Sold during year	24	12.00
	On hand, September 30, 1948	229	
Classic No. 4:	On hand, October 1, 1947	317	
	Sold during year	22	16.50
	On hand, September 30, 1948	295	
Classic No. 5:	On hand, October 1, 1947	547	
	Sold during year	25	31.25
	On hand, September 30, 1948	522	
Classic No. 6:	On hand. October 1, 1947	633	
	Sold during year	25	18.75
	Gratis copy for use by editor	1	
	On hand, September 30, 1948	607	
Classic No. 7:	On hand, October 1, 1947	651	
	Sold during year	44	33.00
	Gratis copy for use by editor	1	
	On hand, September 30, 1948	606	
Classic No. 8:	On hand, March 1, 1948	1,006	
	Sold during year	174	$ 261.00
	Gratis copies sent out for advertising and to members of British Plant Pathology Comm. for aid in selecting M. J. Berkeley papers	11	
	On hand, September 30, 1948	821	
Value of books sent out (Fiscal Year 1947–48)			382.00
Money received during Fiscal Year 1947–48:			
For classics sent out			$ 344.50
On accounts due previous years			4.10
			$ 348.60
Total due on accounts			37.50

Assets:

Cash balance on hand, October 1, 1947	695.25
Receipts during Fiscal Year	348.60
	$1,043.85

Liabilities:

Postage for mailing out classics and for correspondence	$ 23.62
Expense of printing classic No. 8	715.50
Printing and cost of penny postcards for sales promotion	17.50
Bank service charge	1.10
Debit of account for returned check	1.50
	$ 759.22
Cash balance on September 30, 1948	$ 284.63
Total due on account September 30, 1948	$ 37.50

Report of the Placement Committee. During 1948, forty plant pathologists had applications filed with the Placement Committee. Prospective employers listed 53 positions with the Committee. A total of 192 individual applications were sent to employers. Incomplete reports indicate that at least four plant pathologists obtained positions through the efforts of this Committee.

Report of the Public Relations Committee. The Committee members published 17 popular articles on plant disease during the year. The procedure of dealing personally with editors of selected national and regional periodicals has been aided by assigning to each Committee member selected publicity outlets, on a subject-matter and geographic basis. It appears that each Committee member can accomplish more by limiting contributions to a few editors with whom he has developed effective personal contact, than by broader, impersonal activity. The Committee, as in the past, has met its expenses through remuneration for published articles.

Report of the Committee on International Cooperation. Following the effort of the Committee to help our foreign colleagues by sending copies of the 1946 Summary of Nationwide Tests with Newer Fungicides to leading plant pathologists in certain European countries, there was sent to the same addresses, in August, through the embassies or legations in Washington, a copy of the 1947 Summary (Plant Disease Reporter Supplement 176) with the compliments of the Committee.

Because of the very unsettled European situation, however, it seemed desirable to postpone until a more favorable time the project, previously proposed, to publish summaries of investigational results from various countries. During the coming year, however, it is hoped to publish such summaries from Mexico, Argentina, and possibly one or two other Latin-American countries.

Publication of a list of foreign plant pathologists, with special emphasis on those in Latin America, has been proposed. It is hoped that this can be published in CHRONICA BOTANICA and possibly in PHYTOPATHOLOGY. Attempt also is being made to furnish the Committee on Inter-American Scientific Publications (Harlow Shapley, chairman; Mrs. Christina Buechner, executive secretary) a list of some of the most important phytopathological publications in English that should be translated into Spanish. Reciprocally, some of the outstanding Spanish publications may be translated into English. In addition, because of the fact that relatively little plant pathological literature from the United States is available in many Latin American countries, the Committee on International Cooperation hopes to devise some way of stimulating the sending of such literature to outstanding phytopathologists or to libraries.

A few requests from other countries for literature references or other information were referred to our Committee during the year by various agencies, including UNESCO.

Report of Committee on Society Organization. The Committee on Society Organization has had no special problem for consideration this year, except a suggestion from the Secretary that it would be valuable to him to have the official names of the Divisions and a list of their officers for 1948. This information is given below:

Northeastern Division:

President	O. C. Boyd
Vice-President	D. H. Palmiter
Secretary-Treasurer	L. M. Black
Councilor	S. E. A. McCallan

North Central Division:
- President ..... H. C. Young
- Vice-President ..... J. C. Walker
- Secretary-Treasurer ..... M. F. Kernkamp
- Councilor ..... W. F. Buchholtz

Pacific Division:
- President ..... G. W. Fischer
- Vice-President ..... W. C. Snyder
- Secretary-Treasurer ..... G. A. Zentmyer
- Councilor ..... L. C. Cochran

Potomac Division:
- President ..... W. J. Jeffers
- Vice-President ..... C. L. Lefebvre
- Secretary-Treasurer ..... J. B. Demaree
- Councilor ..... Paul R. Miller

Southern Division:
- President ..... I. L. Forbes
- Vice-President ..... L. M. Blank
- Secretary-Treasurer ..... J. A. Lyle
- Councilor ..... S. G. Lehman

Report of the Committee on Plant Disease Prevention. Although the Committee as a unit has not made any concrete contribution, its members have been active. Several members have given considerable time to contacting key men in an attempt to imbue them with the danger of the introduction of new plant pathogens and the need for financial support. The military forces of the United States are fully aware of its existence. Two Committee members were called to Washington for consultation in regard to this matter.

The Committee is also sponsoring a conference at the annual meeting, to which representatives from the different states are invited. The representatives will be given opportunity to express themselves on the general nature of plant disease prevention, with emphasis on the following: (1) means of preventing the introduction of new pathogens or races; (2) methods of detecting new introductions; and (3) organization of plant disease information service, on certain epidemic diseases.

The following resolution was passed unanimously at the conference meeting of the Committee:

The Plant Disease Prevention Committee requests the President of the Society in consultation with the new chairman of this Committee to appoint a group of three to five members, to visit, at least, the Sub-Appropriation Committees of the two houses of Congress, the Budget Bureau, the Secretary of Agriculture, the Secretary of National Defense, and the Chiefs of Bureau of Entomology and Plant Quarantine and Bureau of Plant Industry, Soils, and Agricultural Engineering to present: (1) the urgent need for more effective protection of the major crops and trees against plant diseases, particularly plant diseases not now present in the United States, and (2) to prepare and present to each of the Administrations and groups visited an analytical statement of the critical problems and of the solution proposed.

Report of the Subcommittee on Regulatory Work and Foreign Plant Diseases. It was the understanding when this Committee was appointed that it was to consider problems on which its advice was asked by the federal plant quarantine service. During the year no requests were received.

Report of the Subcommittee on Seed and Plant Material Certification. Although the exact duty of this Committee is not too clear, I believe the members can render valuable service in an advising capacity and also as an aid in bringing together different agencies and organizations which have more or less common interest. Thus, the International Crop Improvement Association asked the chairman to present our viewpoint on diseases in relation to seed certification at their December meeting in Kansas City, Missouri. In addition, the Committee is sponsoring at the annual meeting of The American Phytopathological Society a symposium on "The Nature and Methods of Inspection of Nursery Stock for Importation and Interstate Shipment." Topics for discussion include: (1) viewpoint of state pathologists, nurserymen, plant quarantine service (domestic and foreign), and research workers; (2) irregularities in inspection in different states in relation to interstate commerce and the need for minimum certification standards; and (3) problems involved in the identification of certain diseased nursery stocks.

Report of the Subcommittee on Utilization of Plant Pathologists and Field Facilities in National Emergencies. A report of progress of the Committee on the Utilization of Plant Pathologists and Field Facilities in National Emergencies follows:

1. The Committee has continued with its unfinished efforts of the previous year and has initiated a consideration of Dr. Waldee's suggestion on the utilization of plant pathologists in military government. No final action has been taken on these. It evaluated and approved a suggestion from Dr. Kent on the expansion of the committee's activities to include civilian defense. It sent, through the secretary, to one plant pathologist at each of the experiment stations its form letter of information about Research and Development Units for Reserve Officers. This letter called attention to the opportunity of plant pathologists who are reserve officers to obtain technical assignments in the armed forces through the authority of the Department of Army Circular 127, 1948. It also suggested that the information be brought to the attention of reserve officers at the state experiment stations and U. S. Department of Agriculture.

2. The following recommendations are presented to the Society:

(a) The American Phytopathological Society continues this Committee for 1949

(b) The American Phytopathological Society through some practical means as the "News Letter" inform its members in the United States that the Military Establishment has made provisions for qualified plant pathologists:

(1) to obtain professional and technical commissions in the Officers Reserve Corps under authority of Department of Army Circular 210, dated July 14, 1948, and

(2) if now a reserve officer, to transfer to technical assignments with appropriate promotion under authority of this circular.

(c) The American Phytopathological Society notify the American Institute of Biological Sciences (if and when affiliated with it) that the Committee on Utilization of Plant Pathologists and Field Facilities in National Emergencies exists and suggest that a similar committee be considered for appointment by that organization.

(d) The American Phytopathological Society offers its services (through the Committee on Utilization of Plant Pathologists and Field Facilities in National Emergencies) to the Secretary of National Defense for the purpose of:

(1) studying jointly the offensive and defensive needs and potentialities (that security permits) of personnel trained in the science of plant pathology to the Army, Navy, and Air Force for National Defense, and

(2) evaluating the military duties for which plant pathologists are qualified, and to assist in the drafting of job classification, description, and assignments.

Report of the Committee on Resolutions. The American Phytopathological Society expresses its real appreciation:

To W. C. Price, Chairman, and J. G. Leach, H. G. Guy, Allen Bauer, and O. E. Jennings, members of the committee on local arrangements, for their exacting efforts in assuring the success of the fortieth annual meeting;

To the management and staff of the William Penn Hotel for their considerate and courteous treatment of the Society and its members;

To the following sponsors whose assistance contributed so much to the excellence of the meeting facilities and the annual dinner: Carbide & Carbon Chemicals Corp.; The Dow Chemical Co.; E. I. DuPont de Nemours & Co., Grasselli Chemicals Department; The Harshaw Chemical Co.; Innis, Speiden & Co.; Koppers Co.; Monsanto Chemical Co.; Niagara Chemical Division, Food Machinery Corp.; Phelps Dodge Refining Corp.; Rohm & Haas Co., Agricultural & Sanitary Chemicals Division; Stauffer Chemical Co.; Sindar Corp.; S. W. Clark, Texas Gulf Sulphur Co.; Westinghouse Electric Corp.;

To the Westinghouse Quartet for furnishing the climax to a superb banquet; and

To the members of the Pittsburgh Convention Bureau for their generous supervision of registration.

The members of the Society express their thanks to President Kirby, Vice-President Valleau, and Secretary May for their efforts during the past year, and to Treasurer Richards for his services during the past two years.

Elections and Appointments. A committee of the Council opened and counted the ballots for president, vice-president, and councilor-at-large. W. D. Valleau, C. M. Tucker, and W. H. Tisdale were elected to these respective offices. The Council recommended and the Society approved the appointment of A. E. Dimond as Treasurer for a 3-year term, to succeed M. C. Richards at the close of the fiscal year.

The Council recommended and the Society approved the reappointment of Helen Hart as Editor-in-Chief of PHYTOPATHOLOGY for a 3-year term expiring in 1951. The Society thanks Dr. Hart for her meritorious services and untiring efforts in behalf of the Journal and the Society during the past three years.

The Council recommended and the Society approved the appointment of G. C. Kent as Editor for a 3-year term through 1951, and Harold T. Cook, Berch W. Henry, C. S. Holton, and J. Duain Moore as Associate Editors for 3-year terms, expiring in 1951. L. J. Alexander was reappointed Advertising Manager of PHYTOPATHOLOGY for 1949; L. C. Knorr was appointed Editor of PHYTOPATHOLOGICAL CLASSICS for 1949, and L. J. Tyler, Business Manager; J. C. Walker was appointed representative to the American Institute of Biological Sciences and remains as the Society representative to the Division of Biology and Agriculture of the National Research Council for 1949; R. S. Kirby was appointed as representative on the AAAS Council for 1949–50; Charles Chupp remains as representative on the Board of Editors, American Journal of Botany through 1950; H. H. McKinney was appointed Society representative to the American Type Culture Collection; and H. H. Thornberry was appointed as Society member of the Scientific Liaison and Advisory Board, Quartermaster Food and Container Institute for the Armed Forces.

Reports of Officers, Representatives, and Standing Committees are published on previous pages. Reports of Special and Temporary Committees are not published in the annual report. All committee reports submitted were considered and approved by the Council and accepted by the Society.

The Council recommended and the Society approved the following actions:

1. The Board of Editors is authorized to determine the disposition of the proposed lists of foreign plant pathologists now being considered by the Committee on International Cooperation;

2. continuation is authorized of publication of the NEWS LETTER, and K. W. Kreitlow is reappointed Editor. The unused balance of funds appropriated for 1948 are made available for use in 1949 and an additional sum of three hundred dollars is also made available for the NEWS LETTER in 1949;

3. the Society shall meet with the AAAS in New York City in 1949;

4. the Program Committee is authorized to develop a tentative schedule of meeting for 1950 and 1951. It is suggested that in 1950 the meetings be held either in the South or in the Great Plains and that in 1951 an effort be made to meet with the Entomological Societies, which it is understood now plan to meet in 1951 in the area between Chicago and Pittsburgh. The date of the meetings shall be determined by the Program Committee with preference for early December or early February;

5. it is recommended that the Constitution be amended in prescribed manner to provide for a new class of members to be known known as Emeritus members. It is proposed that the qualifications for Emeritus membership be as follows: (a) any member of the Society, who has been a member for 20 years, may upon retirement from professional duties apply for Emeritus status; (b) an Emeritus member shall retain all rights and privileges as a member of the Society but shall not receive PHYTOPATHOLOGY unless he so desires, in which case he shall receive it at cost;

6. the committee on Society Organization is dismissed with the thanks of the Society. This Standing Committee is discontinued;

7. the Society commends the Membership List Committee for their careful work and the excellence of the List which was published as section 2 of the August 1948 issue of PHYTOPATHOLOGY. The Society thanks S. E. A. McCallan for the preparation of the map showing the geographical distribution of members of the Society. The Membership List Committee is discharged with the sincere thanks and gratitude of the Society.

8. Article 4 of the Standing Rules shall be amended to read as follows: the Secretary, the Treasurer, the President or in his place the Vice-President, are authorized to receive reimbursement from the Society for travel expenses in connection with their attendance at the annual meeting. In addition the Editor-in-chief of PHYTOPATHOLOGY is also authorized to receive reimbursement for travel expenses to the annual meeting.

9. In accordance with previous Society action when affiliation with the American Institute of Biological Sciences was approved, Article I(a) of the Standing Rules shall be changed to read as follows:

(a) The dues for all annual members as defined in Section 2(b), Article II of the Constitution, including subscription to PHYTOPATHOLOGY, shall be $7.00 a year, payable by February 1. The Business Manager is authorized to discontinue sending the Journal to members whose dues have not been paid by this date.

10. The Treasurer is authorized to pay $850.00 to the American Institute of Biological Sciences as the membership fee for the Society for 1949.

11. In accord with common procedure, the Treasurer is authorized to pay to the new Treasurer and Business Manager of PHYTOPATHOLOGY necessary travel expenses incurred incident to assuming his new duties.

12. A. J. Riker is delegated to act with the President, Business Manager of PHYTOPATHOLOGY, and the Editor-in-chief with respect to publication problems of PHYTOPATHOLOGY.

The following resolution was introduced from the floor and after discussion was approved.

WHEREAS: The Plant Disease Reporter, issued by the Division of Mycology and Disease Survey, has proved to be invaluable in advancing plant disease research and extension work in this country and

WHEREAS it is believed by many that its full value as a reference source has not been realized owing to the somewhat bulky and fragile form imposed by the mimeograph process now in use and

WHEREAS if either a photo offset or a printing process could be employed, using a suitable format, its value for reference and research would be improved

BE IT RESOLVED THEREFORE that The American Phytopathological Society urge that the Plant Disease Reporter be published in more permanent form for reference purposes, if this can be done without sacrificing promptness of issue which has contributed so much to its usefulness

BE IT FURTHER RESOLVED that copies of this resolution be sent to the Secretary of Agriculture, the Administrator of the Agricultural Research Administration, the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Head of the Division of Mycology and Disease Survey.

Curtis May, Secretary

MINUTES OF THE BUSINESS MEETING, NORTHEASTERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, DECEMBER 6, 1948

President O. C. Boyd presided. The minutes of the business meeting of July 23, a brief account of the summer meeting at Ithaca and Geneva, New York, and the Treasurer's report were read by the Secretary-Treasurer, and approved by the membership. Dr. James G. Horsfall, chairman of the Committee on Nominations, placed the following names before the members: President, D. H. Palmiter; Vice-President, E. M. Stoddard; Secretary-Treasurer, L. M. Black; and Councilor, S. E. A. McCallan. No further nominations coming from the floor, it was moved, seconded, and carried that the Secretary record a unanimous ballot for the above candidates. Discussion regarding the time and place of the next meeting revealed that a majority favored a summer meeting without papers. A motion to leave further details of the summer meeting to the Executive Committee was passed. Dr. D. H. Palmiter proposed that "Inasmuch as the officers of the parent society made all arrangements for the joint meeting of the Northeastern Division with the parent society: be it resolved that the Northeastern Division express its thanks to the President, Secretary, and other officers of The American Phytopathological Society." The resolution was adopted and the Secretary instructed to forward copies to President Kirby and Secretary May.

L. M. Black, Secretary-Treasurer